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REVISTA DE FARMACOLOGÍA DE CHILE

Órgano oficial de la Sociedad de Farmacología de Chile

¡BIENVENIDOS AL CONGRESO ANUAL DE LA SOCIEDAD DE FARMACOLOGÍA DE CHILE!

CONCEPCIÓN 2019

EL EVENTO CIENTÍFICO CONTARÁ CON LA PARTICIPACIÓN DE 5 CONFERENCISTAS INTERNACIONALES, 70 EXPOSITORES EN SIMPOSIOS Y MINI SIMPOSIOS, JUNTO A CERCA DE 200 JÓVENES CIENTÍFICOS QUE EXHIBIRÁN LOS AVANCES OBTENIDOS EN SUS DIFERENTES LÍNEAS DE INVESTIGACIÓN.

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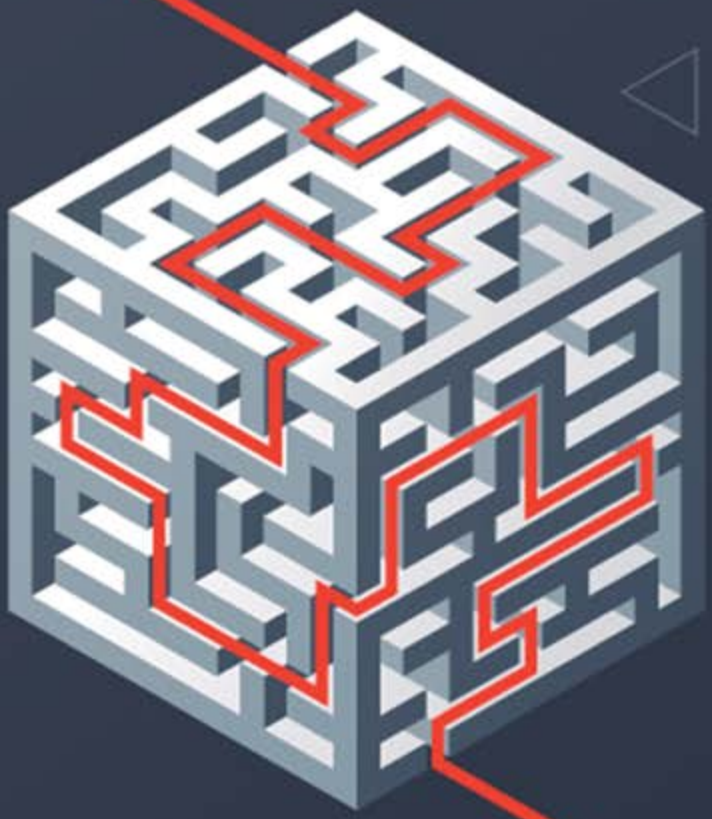

EL CAMINO HACIA LA MEDICINA PERSONALIZADA

Descubre el camino a seguir para el tratamiento de sus pacientes con cáncer, utilizando la detallada información del perfilamiento genómico integral de Foundation Medicine[1-6]

Directiva

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La Revista de Farmacología de Chile tiene un panel de editores conformado por connotados farmacólogos nacionales que son miembros de la Sociedad de Farmacología de Chile y académicos de las principales universidades chilenas.

Comité Editorial

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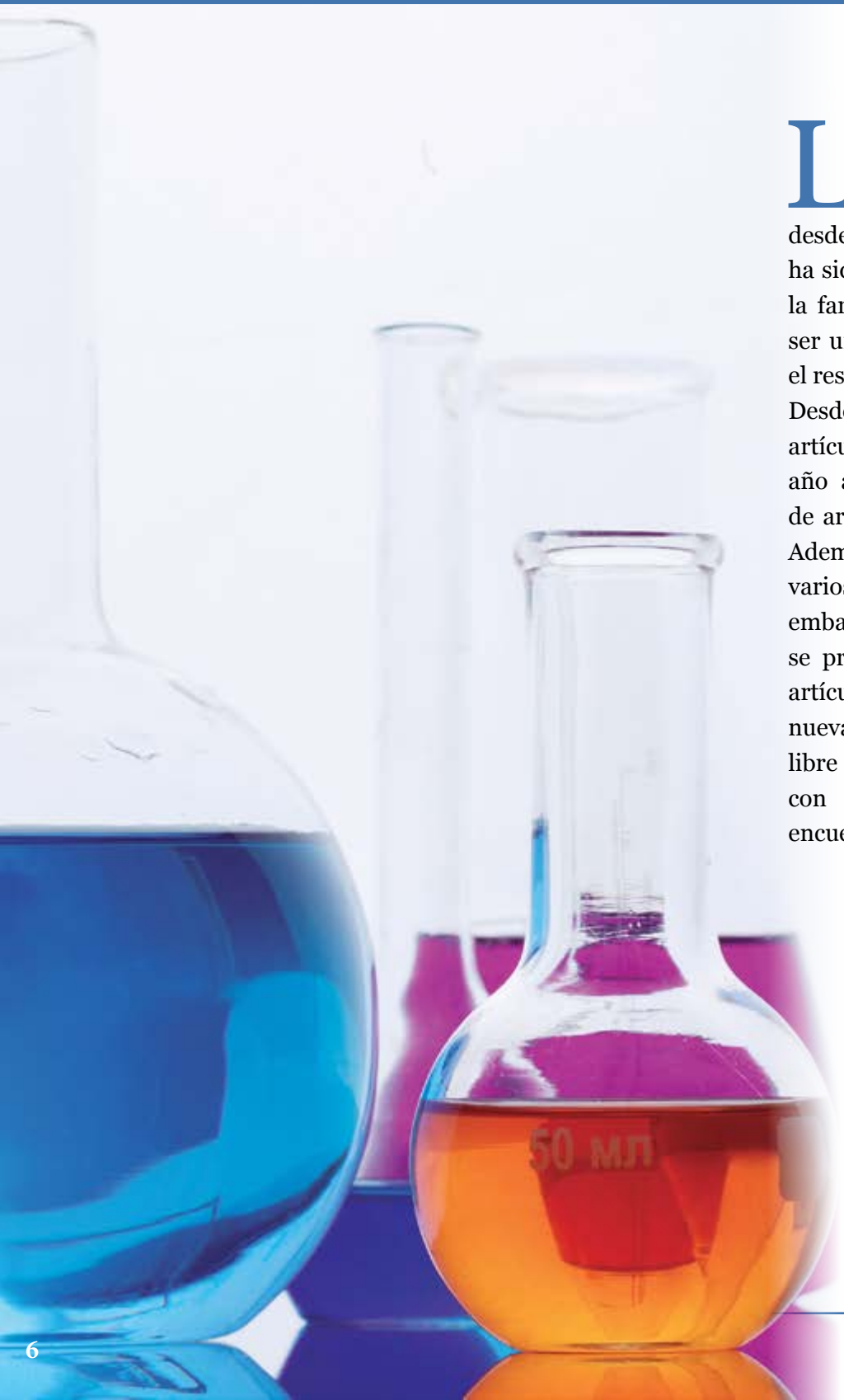


La Revista de Farmacología de Chile es una revista de información científica vigente desde el año 2008. Su objetivo inicial ha sido ser un órgano de difusión de la farmacología en Chile para luego ser una plataforma de difusión para el resto de Latinoamérica.

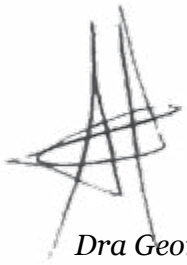
Desde sus comienzos, la recepción de artículos científicos fue aumentando año a año, llegando a un máximo de artículos publicados el año 2014. Además, hemos logrado contar con varios artículos internacionales. Sin embargo, desde esa fecha en adelante se produjo una disminución en los artículos recibidos que coincidió con nuevas revistas de la disciplina de libre acceso y una gran competencia con revistas del área que ya se encuentran indexadas.

Editorial

En este sentido, se nos ha planteado un gran desafío para los próximos años en pro de revertir esta tendencia. Por un lado, debemos aumentar la difusión y distribución de la revista a nivel latinoamericano y, asimismo, estamos evaluando la posibilidad de asociarnos a una casa editorial internacional y cambiar el idioma de publicación de nuestra Revista.

En el volumen de este año, hemos logrado volver a aumentar el número de Revistas publicadas con respecto al año anterior. En este número de la Revista se publican dos artículos originales internacionales (Cuba y España) y dos artículos nacionales. Además, teniendo en cuenta que este número corresponde al XLI Congreso Anual de la Sociedad de Farmacología, se publican los resúmenes correspondientes a las conferencias, simposios y paneles que serán presentados del 4 al 8 de noviembre en la sede del congreso, Universidad de Concepción.

Nuevamente invitamos a todos a ser parte del crecimiento de la Revista de Farmacología de Chile y lograr difundir información científica del área a nivel internacional.



Dra Georgina Renard
Co - Editora

Directora por Santiago Sociedad de Farmacología de Chile

"Por un lado, debemos aumentar la difusión y distribución de la revista a nivel Latinoamericano y, asimismo, estamos evaluando la posibilidad de asociarnos a una casa editorial internacional y cambiar el idioma de publicación de nuestra Revista".

La sociedad y Sofarchi: un vínculo que se expande

Estimadas socias y socios:

"En esta conversación, le expresamos al Ministro la total disposición de la Sofarchi para ser un modulador positivo en esta tarea, y pusimos a su disposición los diferentes canales de comunicación de nuestra sociedad para ayudar en ese objetivo".

Estimadas socias y socios, junto con extenderles un cordial saludo desde nuestra directiva, les invitamos a leer nuestro nuevo número de la revista, Editado con el incansable trabajo del Dr. Ramón Sotomayor como editor en jefe, Dra. Georgina Renard como co-Editora, Fabiola Valdebenito, nuestra periodista, y todos quienes de una u otra forma colaboran para que la revista siga activamente siendo un canal de comunicación con ustedes y la sociedad. En este número podrán encontrar el informe de algunas de las más recientes actividades donde nuestra sociedad ha estado presente, actualidad científica, artículos científicos originales y revisiones, entre otros.

Respecto a la actualidad científica nacional, durante el primer semestre, hemos participado en una interesante reunión con el Ministro de Ciencia Dr. Andres Couve, quien nos contó los avances que su ministerio ha experimentado, y las metas de mediano y largo plazo que se han impuesto, como el acercar la actividad científica al centro de atención de la sociedad. En esta conversación, le expresamos al Ministro la total disposición de la SOFARCHI para ser un modulador positivo en esta tarea, y pusimos a su disposición los diferentes canales de comunicación de nuestra sociedad para ayudar en ese objetivo.

En este plan de acercar la ciencia a la sociedad, la SOFARCHI realizó la pasada reunión de la Directiva en la ciudad de Coquimbo. Esta reunión fue realizada en la Facultad de Medicina de la Universidad Católica del Norte, reuniéndose con nosotros el decano de la facultad con quien discutimos nuevas oportunidades de integración. Además, como actividad de divulgación a la sociedad se realizó una charla científica a alumnos del Colegio Alemán de La Serena.

A nivel internacional, 4 miembros de nuestra sociedad nos representaron en la Reunión Anual de la Sociedad Española de Farmacología, realizada en el pasado mes de Julio en La Palma de Gran Canaria, España. En este congreso, donde además participaron representantes de las sociedades de farmacología de Alemania y Holanda, pudimos compartir e intercambiar experiencias y generar colaboraciones entre nuestras sociedades que esperamos den sus frutos en el mediano plazo.

Por último, estamos trabajando sin pausa, y con nuestros mejores esfuerzos, para organizar nuestra próxima reunión en Noviembre, la que contará con importantes expositores nacionales e internacionales en 7 conferencias plenarias, 10 simposios, comunicaciones orales, comunicaciones en póster y actividades satélites que complementaran una intensa jornada científica de 5 días.

A nivel personal, tengo el secreto anhelo que puedan re-descubrir la belleza del Campus Concepción, en el centenario de la Universidad de Concepción, y sus alrededores, y les reitero mi más ferviente invitación para que nos acompañen en esta jornada científica que nos prepara varias sorpresas.

Un atento saludo a todos Uds. y disfruten de este nuevo número de nuestra nueva revista

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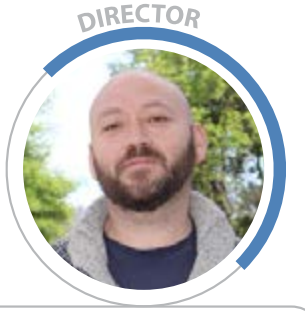

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"El encuentro, realizado en el Palacio de La Moneda, contó con la participación de cerca de 25 organizaciones dedicadas a la investigación y promoción de diversas áreas del saber, entre ellas, la Sociedad de Farmacología de Chile".

Sociedades científicas se reúnen con Ministro Andrés Couve para conocer lineamientos para promover investigación

Con el objetivo de entregar detalles sobre el estado de avance de la instalación del Ministerio de Ciencias, Tecnología e Innovación, además de exponer sobre las directrices que se van a implementar para el desarrollo de investigación, se realizó una reunión entre el jefe de la cartera, Andrés Couve, y los representantes de las principales sociedades científicas del país.

El encuentro, realizado en el Palacio de La Moneda, contó con la participación de cerca de 25 organizaciones dedicadas a la investigación y promoción de diversas áreas del saber, entre ellas la Sociedad de Farmacología de Chile Sofarchi. Su presidente, Jorge Fuentealba, indicó que "la actividad ha sido muy productiva, en el sentido de poder avizorar cuáles son las etapas que ha cumplido el Ministerio y cuáles son las que quedan dentro de la instalación de esta importante repartición del estado que va a potenciar la investigación científica".

Uno de los ejes de la reunión giró en torno a las estrategias que las organizaciones podrían desarrollar en conjunto para contribuir a potenciar el desarrollo de la ciencia en Chile. En este sentido, Fuentealba agregó que "la principal preocupación que manifesté al ministro dice en relación a cómo las 20 o 25 sociedades que estábamos en la mesa podíamos contribuir a potenciar el desarrollo de la ciencia en términos de darle difusión, de demostrar la calidad de la investigación que se hace en los distintos ámbitos, desde las ciencias sociales hasta las ciencias naturales y exactas", expuso.

El líder de la Sofarchi añadió que "el ministro dijo que confía plenamente en que vamos a ser actores activos y relevantes en la difusión y fortalecimiento de los conceptos de ciencia y de generación de conocimientos", puntualizó.

"La principal preocupación que manifesté al ministro dice en relación a cómo las 20 o 25 sociedades que estábamos en la mesa podíamos contribuir a potenciar el desarrollo de la ciencia en términos de darle difusión, de demostrar la calidad de la investigación que se hace en los distintos ámbitos, desde las ciencias sociales hasta las ciencias naturales y exactas", Jorge Fuentealba A.

Hasta La Serena, en la región de Coquimbo, se trasladó la directiva de la Sociedad de Farmacología de Chile, con el objeto de realizar diversas actividades orientadas a fortalecer el desarrollo de este organismo científico en regiones y a motivar la incorporación de nuevos socios.

La visita estuvo encabezada por su presidente, Jorge Fuentealba, acompañado por el Vicepresidente de la entidad, Ramón Sotomayor, junto al académico de la UCN y Secretario General de Sofarchi, Claudio Coddou y las directoras del organismo, Georgina Renard y Viviana Noriega.

El equipo sostuvo un encuentro con el Decano de la Facultad de Medicina de la Universidad Católica del Norte, Osvaldo Iribarren, quien pudo conocer detalles sobre las principales líneas de trabajo que está desarrollando la organización durante este año, lo que permitió sentar las bases para próximas acciones conjuntas.

"La idea de reunirnos en Coquimbo es potenciar las actividades en regiones y fortalecer el interés de los científicos de esta zona del país para integrarse a Sofarchi y colaborar, de esta forma, al desarrollo de la disciplina", señaló Jorge Fuentealba. Agregó que "actividades como estas refuerzan nuestro rol social, dando a conocer la ciencia y la farmacología. Por eso, estamos muy contentos con la recepción que hemos tenido de parte del Decano, ya que es importante contar con ese entusiasmo y creo firmemente que será un motor de empuje para esta iniciativa en la región".

En este sentido, el decano de la Facultad de Medicina de la Universidad Católica del Norte, Osvaldo Iribarren, comentó que "la UCN sustenta su funcionamiento y su desarrollo en tres pilares: la docencia, la investigación y

Directiva de Sofarchi visita Coquimbo para promover la participación de socios

la vinculación. Para nosotros, como Facultad de Medicina, tenemos que ir empujando la frontera del conocimiento cada vez mas y una sociedad científica como Sofarchi representa exactamente está construcción de vínculos"
En este sentido, agregó que esta asociación "nos permite ir abriendo otros lazos sutiles, pero muy firmes, con el resto de las universidades, porque la Sofarchi está en la Universidad de Concepción, en la de Valparaíso, en la de Santiago, etcétera; así es que para nosotros no puede ser mejor y más bienvenida", manifestó.
Respecto a las actividades a realizar en conjunto, Irribarren indicó que "me invitaron a la inauguración del Congreso de Sofarchi que se realizará en noviembre próximo en Concepción, lo cual me parece muy bien porque está en perfecta sintonía con la vinculación cada vez más fuerte que pretendo hacer con el ambiente académico", expuso.

El presidente de Sofarchi, Jorge Fuentealba, se reunió con el Decano de la Universidad Católica del Norte, Doctor Osvaldo Iribarren; para exponer sobre los principales lineamientos de trabajo de la organización farmacológica. Con ello, se busca sentar las bases de futuras acciones conjuntas.

Ciencia más cerca

Otra de las actividades en las que participó la directiva se desarrolló en el Colegio Alemán de La Serena, donde cerca de 70 escolares de octavo básico a segundo medio asistieron a la charla "Mirando la naturaleza, el laboratorio maestro".
"Tuvimos una grata jornada con niños y jóvenes que nos dieron una muy buena recepción, fueron muy interactivos, hicieron muchas preguntas. Fue muy grato sentir que en Coquimbo hay un sustrato para poder desarrollar y potenciar esta área, que permitiría favorecer el desarrollo de la biomedicina en la región", explicó Fuentealba.
A través de la exposición, los escolares aprendieron sobre el valor de la observación como mecanismo para entender diversos procesos de la naturaleza, desde donde la humanidad ha extraído fármacos desde tiempos inmemoriales. El investigador agregó que "hoy en día esos principios farmacológicos son estudiados científicamente y aplicados a la biomedicina, para transformarlos en medicamentos que permiten mejorar la calidad de vida de millones de personas en todo el mundo y que tienen su origen en este laboratorio maestro que es la naturaleza".
Otro de los presentes en la jornada fue el secretario de la organización científica, Claudio Coddou, quien comentó que "es muy importante tener estos espacios, porque gracias al link con la Sociedad de Farmacología estamos promoviendo la Universidad y generando interés de los estudiantes hacia este mundo de la ciencia".

Delegación de la Sofarchi participó en el Congreso de la Sociedad Española de Farmacología

Con la colaboración internacional de las asociaciones farmacológicas alemana, holandesa, irlandesa y chilena, se efectuó el 39° Congreso Anual de la Sociedad Española de Farmacología (Socesfa), realizado por primera vez en Las Palmas de Gran Canaria. Más de 60 expositores de diversas partes de globo divididos en 13 sesiones de simposios, compartieron sus conocimientos con farmacólogos, científicos, médicos, profesionales de área de la salud, pacientes y representantes de la industria farmacéutica, convirtiendo a este encuentro en uno de los más relevantes del planeta en su tipo.

La delegación de la Sofarchi estuvo integrada por los doctores Jorge Fuentealba y Leonardo Guzmán, de la Universidad de Concepción; junto a Ramón Sotomayor, de la Universidad Católica de Valparaíso y Guillermo Díaz, de la Universidad de Chile.

Respecto a esta participación, el presidente de la Sofarchi, Jorge Fuentealba, indicó que "como directiva nos parece muy relevante que una Sociedad tan grande como la española nos reconozca y nos trate como pares, invitándonos a participar en su Congreso". El directivo agregó que "fue una actividad muy interesante, científicamente de alto nivel y con bastantes oportunidades para el intercambio, para la vinculación y para el desarrollo de articulaciones de estudiantes y de trabajos de investigación", expuso.

Los representantes nacionales realizaron cuatro exposiciones plenarias dentro de los simposios, la primera a cargo del doctor Guillermo Díaz, quien se refirió al rol del fibroblasto cardíaco en procesos inflamatorios y la interacción con células inmunes.

En tanto, el doctor Ramón Sotomayor abordó la programación de neuronas dopaminérgicas por exposición temprana a hormonas sexuales y su posible efecto en la vulnerabilidad ante la drogadicción. Además, valoró la posibilidad que otorgan estos eventos internacionales para potenciar el intercambio de experiencias en nuestro país, "nos contactamos con Michael Spedding, secretario gene-

ral de la International Union of Basic & Clinical Pharmacology para invitar a la representante de la UPHAR el próximo año a Chile. Además, logramos confirmar la participación de la Doctora Concha Peiró, de la Universidad Autónoma de Madrid y miembro de la directiva de la Socesfar a nuestro próximo Congreso Anual de Sofarchi que se realizará en Concepción durante este año", detalló el doctor Sotomayor.

Aporte científico

La farmacología es entendida como una ciencia necesariamente interdisciplinaria, por lo que en el Congreso se trataron temáticas tan diversas como cáncer, receptores, diseño de drogas, farmacogenética, farmacología clínica en áreas de endocrinología, gastroenterología y respiratorios; además de farmacología del dolor y la inflamación, entre otros.

El equipo de académicos de la Universidad de Concepción presente en este 39° Congreso de la Socesfar, participó tanto en el área de envejecimiento, considerando desde los mecanismos hasta las perspectivas farmacológicas; como en nuevos avances en neurofarmacología.

En el desarrollo de este último tema, abordado en un simposio en el que expusieron otros tres investigadores; participó el Doctor Leonardo Guzmán con su charla titulada "efectos de los dendrímeros PAMAM en la funcionalidad neuronal In Vitro". El investigador detalló que en la ocasión "se mostraron avances en neurofármacos que se están desarrollando para ayudar en procesos de adicciones; también se mostraron investigaciones sobre intervenciones para la liberación de neurotransmisiones y, por mi parte, mostré cómo los nanotransportadores podrían ayudar a la entrega de fármacos en sitios sinápticos", detalló.

En tanto, Jorge Fuentealba presentó "Sobreexpresión de P2xr contribuye a la toxicidad por Beta Amiloides: nuevo blanco farmacológico para la AD", en la que expone parte de su investigación orientada al análisis de los mecanismos celulares y moleculares que inducen el desarrollo de enfermedades neurodegenerativas, especialmente la Enfermedad de Alzheimer.

Cuatro representantes de la organización científica chilena fueron invitados por la Socesfar para exponer parte de sus investigaciones. En la actividad también participaron sociedades farmacológicas de Holanda, Irlanda y Alemania.

Cien años de Química

"Hace exactamente 100 años tenía lugar la primera clase del curso de Farmacia, dictada por don Salvador Gálvez Rojas a 28 estudiantes, todas ellas mujeres, constituyéndose en una de las primeras carreras universitarias en el sur de Chile que ofrecía una opción de educación superior para las mujeres del país".

La conmemoración de los 100 años de la Carrera de Química y Farmacia, de la Facultad de Farmacia de la Universidad de Concepción en este año 2019, constituye uno de los más importantes hitos en la historia de la también centenaria Universidad de Concepción y es especialmente significativa para todos quienes hemos tenido el privilegio de realizar nuestros estudios universitarios en esta Alma Mater.

Hace exactamente 100 años tenía lugar la primera clase del curso de Farmacia, dictada por don Salvador Gálvez Rojas a 28 estudiantes, todas ellas mujeres, constituyéndose en una de las primeras carreras universitarias en el sur de Chile que ofrecía una opción de educación superior para las mujeres del país.

Cien años desde aquel sencillo pero visionario comienzo en que nacía la carrera de Química y Farmacia y al mismo tiempo, la entonces, Escuela de Farmacia, cuando la creación de la carrera - la segunda históricamente en el país - fue promovida con decisión por los fundadores desde el momento en que el Comité Pro Universidad y Hospital Clínico creado en 1917 empieza a definir la forma en que la novel Casa de Estudios iniciaría sus actividades.

y Farmacia UdeC

Respondiendo a una sentida necesidad, no sólo de la región sino del país, pues con su creación se buscaba el mejoramiento sanitario de la población.

Cien años después, la Facultad de Farmacia ha formado más de 2.800 profesionales Químicos Farmacéuticos, que se desempeñan en los distintos ámbitos del ejercicio profesional, representando un importante aporte al desarrollo del país, particularmente en el sector sanitario, el sector productivo, la academia y también en la política universitaria y ciudadana.

En este contexto es relevante destacar la figura de Ligia Gargallo González, químico farmacéutica, académica e investigadora, Premio Nacional de Ciencias Naturales 2014 y Premio L'Oreal UNESCO 2007 para Mujeres en Ciencias. Del mismo modo, reconocer el esfuerzo, compromiso, dedicación y liderazgo de recordados maestros y profesores.

Durante estos 100 años son muchísimos los hitos que marcan la historia de la Carrera de Química y Farmacia: la Farmacia Modelo en 1920, un centro de práctica, único e innovador para la época, con un fuerte sentido social; la estructura y organización definitiva como Facultad en 1927; el surgimiento de nuevas iniciativas de formación profesional como la creación de las carreras de Bioquímica en 1957 y de Nutrición y Dietética en 1975; las fiestas universitarias, juegos florales y carnavales de antaño; la difícil y anhelada Autorización Ministerial para que la Universidad de Concepción pudiera otorgar los títulos de Químico Farmacéutico y de Bioquímico hacia fines de 1974; la ampliación del actual edificio y el surgimiento del Postgrado. En este ámbito, la Facultad de Farmacia ofrece actualmente, un programa de doctorado, 3 programas de magíster y el recientemente creado programa de Especialización en Farmacia Clínica. Sumando también Cursos, Diplomas y Diplomados.

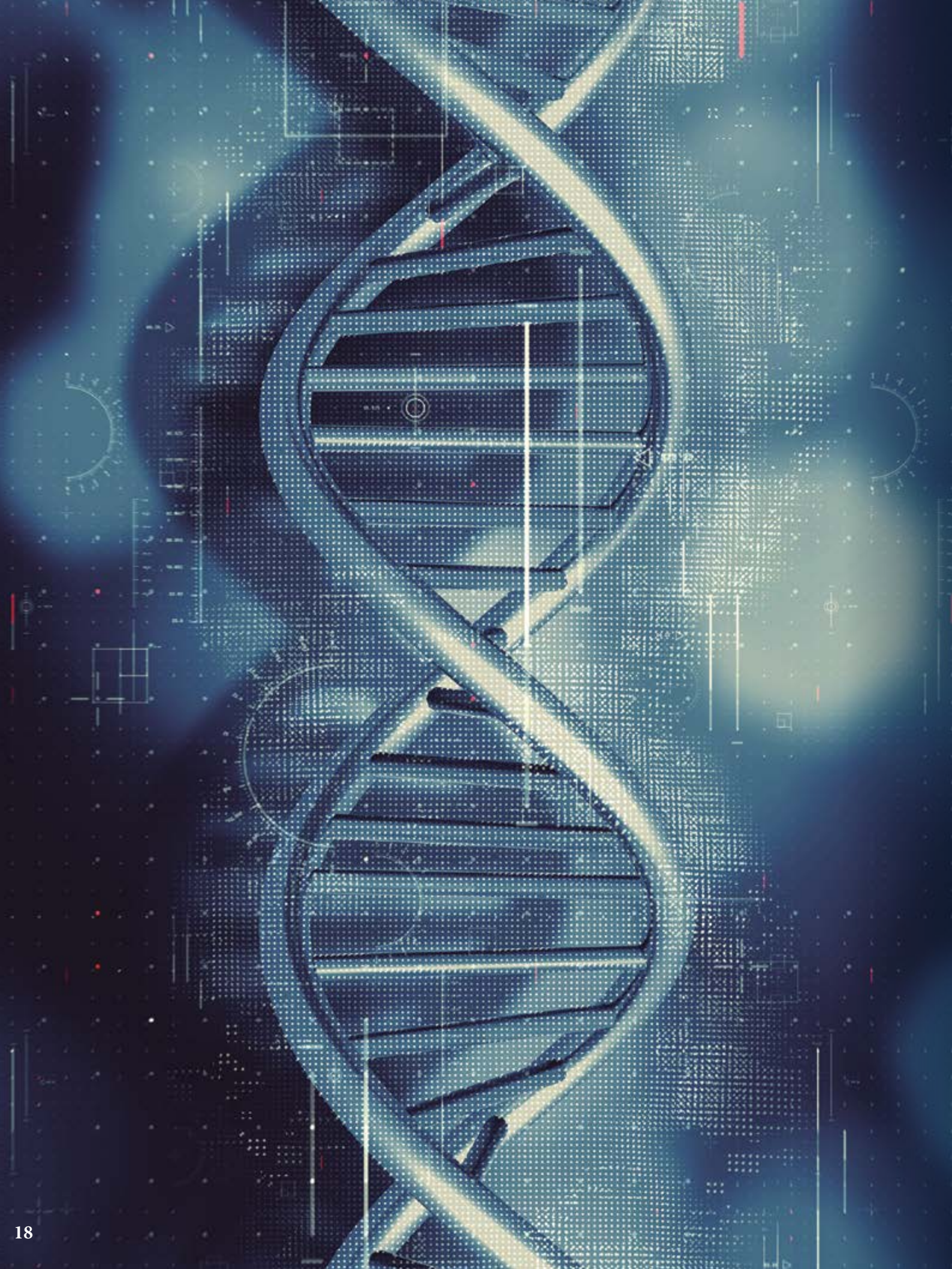
La investigación como pilar para la generación de nuevo conocimiento también ha sido un área destacada en el quehacer de la Facultad de Farmacia, reflejado en publicaciones en revistas de corriente principal y adjudicación de proyectos de investigación con financiamiento externo (Fondecyt, Fondef, entre otros). Desde 2015 a la fecha, la Facultad ha adjudicado 4 iniciativas Fondequip para la adquisición de equipamiento mediano que ha significado para la Universidad de Concepción la atracción de más de 1.000 millones de pesos para investigación, permitiendo el desarrollo y crecimiento de la Facultad y la institución, además de mayores posibilidades de formación terminal para estudiantes de pre y postgrado.

Muchos desafíos y tareas se proyectan para la Facultad, sin embargo, es necesario destacar nuevamente el perenne sello UdeC del profesional químico farmacéutico, esa característica tan propia que permite reconocernos con nuestros pares cualquiera que sea el área laboral y lugar en que nos encontremos, haciendo que nuestros profesionales sean preferidos y respetados.

La invitación es a seguir construyendo y nutriendo con orgullo, respeto, empatía, comportamiento ético y dedicación esta hermosa profesión farmacéutica, para proyectarla exitosa hacia el futuro.

Dr. Ricardo Godoy Ramos
Decano Facultad de Farmacia
Universidad de Concepción

"Cien años después, la Facultad de Farmacia ha formado más de 2.800 profesionales Químicos Farmacéuticos, que se desempeñan en los distintos ámbitos del ejercicio profesional, representando un importante aporte al desarrollo del país, particularmente en el sector sanitario, el sector productivo, la academia y también en la política universitaria y ciudadana".



Formando profesionales en las grandes líneas de desarrollo científico básico y clínico de la farmacología

La necesidad de contar con especialistas que comprendieran en profundidad las bases modernas de la farmacología del Sistema Nervioso y que, además, adoptaran conceptos biomédicos relacionados con la farmacología molecular y clínica, motivó en 2012 a un equipo de académicos de la Facultad de Ciencias de la Universidad de Valparaíso a generar nuevas instancias de formación continua.

Fue así como el Doctor Ramón Sotomayor, académico del Instituto de Fisiología de la Facultad de Ciencias de esta casa de estudios, encabezó la idea de promover el desarrollo de un programa de postgrado en Neurofarmacología. El investigador, quien actualmente es el coordinador de esta instancia, comentó que "este curso se realiza todos los años en la Universidad de Valparaíso y ha sido muy bien evaluado en cuanto a los contenidos que se imparten, así como también por las habilidades experimentales que adquieren los estudiantes durante el transcurso del programa"

Agregó que el curso está orientado tanto a profesionales del área clínica, como neurólogos, neurocirujanos, psiquiatras y psicólogos, entre otros; como a especialistas en neurociencia, farmacología y biomedicina.

Según indicó el Doctor Sotomayor, esta séptima versión "cuenta con estudiantes del Doctorado en Ciencias, mención Neurociencias y del Magíster en Ciencias Biológicas, mención Neurociencias de la Universidad de Valparaíso. Además, hay estudiantes profesionales, incluso, en esta oportunidad contamos con un estudiante polaco que es médico".

El Doctor Sotomayor destacó también la amplia presencia de la Sociedad Chilena de Farmacología en esta instancia, "este curso ha sido patrocinado desde su origen por la Sofarchi, lo que nos ha permitido contar con la participación de profesores de alto nivel provenientes de destacadas Universidades del país".

En este sentido, indicó que "gracias a esta alianza hoy contamos con una decena de académicos que son socios titulares de la Sofarchi, quienes integran del staff de docentes de este curso". Entre ellos, se cuentan los doctores Jorge Fuentealba (Universidad de Concepción), Javier Bravo (Pontificia Universidad Católica de Valparaíso), Claudio Coddou (Universidad Católica del Norte) y Georgina Renard (Universidad de Santiago), entre otros.

Alumnos exitosos

Diversas son las habilidades y conocimientos que los alumnos de este curso de Neurofarmacología han obtenido generación tras generación. A través de una metodología que incluye clases magistrales y seminarios de discusión, se han formado profesionales con pensamiento crítico, capaces de analizar las grandes líneas de desarrollo científico básico y clínico

INSTITUTO DE Fisiología
Facultad de Ciencias Universidad de Valparaíso

Desde hace siete años, este programa prepara con conocimiento teórico y habilidades experimentales tanto a profesionales del área clínica, como a especialistas en neurociencia, farmacología y biomedicina.

que han permitido tanto la generación de los tratamientos farmacológicos actuales, como su evolución y proyecciones futuras.

Junto a ello, la propuesta académica de este programa permite a los alumnos desarrollar trabajos experimentales originales que contribuyen a la generación y desarrollo de tratamientos farmacológicos del sistema nervioso.

Estos y otros conocimientos teóricos y prácticos entregados en el programa de Neurofarmacología, le han entregado herramientas para avanzar en sus investigaciones a la estudiante de segundo año del Magíster en Ciencias Biológicas, mención Neurociencias de la Universidad de Valparaíso, Victoria Collio,

"Estoy haciendo mi tesis sobre el efecto del consumo de dieta alta en grasa en algunos núcleos, como el septum lateral cerebral, en ratas, para evaluar el impacto en el control de la comida, del hambre y de la saciedad", indicó la pedagoga en biología de la Pontificia Universidad Católica de Valparaíso. Hasta ahora, los resultados obtenidos por Victoria Collio han determinado que "aquellas ratas que ingirieron dietas altas en grasa durante gran parte de su vida, a diferencia de las control, siguen prefiriendo esta dieta en grandes proporciones. Hay una diferencia bien importante"

Respecto al aporte que ha sido el programa de Neurofarmacología en el desarrollo de su investigación, sostuvo que "el curso entrega una base neurobiológica en todos los ámbitos, que van desde las adicciones, el movimiento, la fisiología, lo molecular, etcétera. El ramo de Estructura y Función del Sistema Nervioso, por ejemplo, es súper importante y exigente".

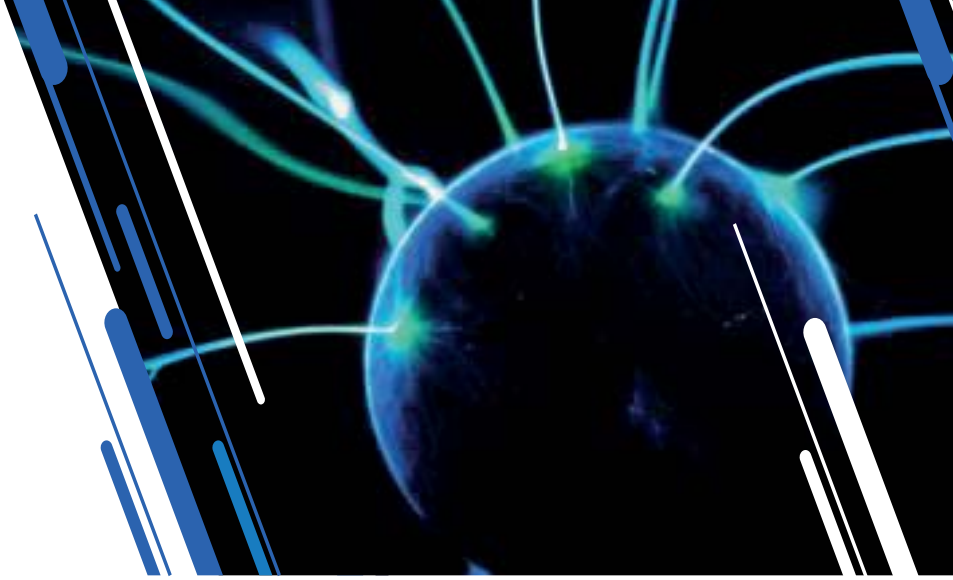
La séptima versión del curso de Neurofarmacología tiene una duración de 18 semanas y considera un total de 162 horas semestrales.

Mayor información:

Sra. Francisca Ramírez Coordinación de Postgrado, Facultad de Ciencias, Universidad de Valparaíso Avda. Gran Bretaña #1111, Playa Ancha, Valparaíso. Fono +56 32 2508000 francisca.ramirez@uv.cl ; postgrado.ciencias@uv.cl

MAGISTER EN CIENCIAS BIOLÓGICAS

MENCIÓN NEUROCIENCIAS



Comisión Nacional de Acreditación
CNA-Chile
MAGÍSTER ACREDITADO
POR 7 AÑOS HASTA JUNIO DE 2026.
COMISIÓN NACIONAL DE ACREDITACIÓN.

Información General

El Programa tiene una duración de 2 años y considera una dedicación de media jornada (22 horas semanales), en modalidad teórico-práctico. Primer año: Actividades y Cursos Obligatorios y Electivos, tales como, Organización General y Desarrollo del Sistema Nervioso, Fisiología Neuronal y Transmisión Sináptica, Fisiología Sensorial, Movimiento y Control Motor, Neuropatología, Metodología Experimental en Neurociencia, Microscopía, Biofísica, Bioestadística, Neurociencia Computacional, Unidades de Investigación, Elaboración del Proyecto de Tesis. Segundo Año: destinado al desarrollo de la Tesis de Grado. El programa podría realizarse en un año, sí el alumno realiza su tesis en los laboratorios del claustro académico interno, demuestra dedicación exclusiva y jornada completa, y si cumple con los requisitos académicos de aprobación de cursos y proyecto de tesis en los tiempos establecidos.

Postulaciones en:

http://postgrados.uv.cl/index.php/magister/neurociencias.

Convocatoria: **Abierta**

Fecha de Cierre **30 de Noviembre 2019**

Horario de Clases

Primer año:

Jueves y Viernes de 10:00 a 17:30 horas

Segundo año:

Desarrollo de Tesis, se acuerdan horarios con el Profesor Tutor.

Línea de investigación

- Estructura y Función de Canales de Iones
- Neurosecreción y Comunicación Celular
- Transmisión y Plasticidad Sináptica
- Neurofisiología Sensorial
- Genética y Desarrollo del Sistema Nervioso
- Neurociencia de Sistemas y Comportamiento
- Bioinformática y Biomatemática en Neurociencia

Información e inscripciones

Mayor información y entrega de antecedentes, dirigirse a secretaria del programa:

postgrado.ciencias@uv.cl
32-2508400 / 32-2508402

Prof. Agustín Martínez C.
director: agustin.martinez@uv.cl

Facultad de Ciencias, Universidad de Valparaíso, Gran Bretaña 1111, Playa Ancha, Valparaíso.

Magíster en Neurobiología:
Formando especialistas en

El programa de la Facultad de Ciencias Biológicas de la UdeC proporciona un contenido académico orientado a la formación de especialistas en esta área, poniendo énfasis en el aprendizaje práctico de técnicas, protocolos, procedimientos de experimentación en neurobiología celular y molecular, además de estudio de comportamiento animal.

investigación experimental

Las bondades del ajo han sido ampliamente valoradas desde tiempos remotos. Es considerado uno de los productos alimenticios más completos en la naturaleza gracias a la larga lista de vitaminas y minerales que posee, que le otorgan comprobadas propiedades cardioprotectoras, hipoglicemiantes y antioxidantes.

A este listado de beneficios se agrega una nueva cualidad descubierta recientemente por un equipo de investigadores de la Universidad de Concepción, quienes analizaron las capacidades neuroprotectoras de una variedad fermentada del Allium ampeloprasum o ajo chilote, una especie endémica de la Isla Grande. Según la bióloga que encabeza el estudio, Javiera Gavilán, este alimento cambia tras de ser sometido a condiciones específicas de calor y humedad, lo que le otorga su característico color oscuro y potencia sus propiedades. "Nos dimos cuenta de que su composición química es distinta al ajo que comemos normalmente, porque adquiere nuevos compuestos sulfurados. Desde ahí partió el interés de probar su efecto neuroprotector en modelos de Alzheimer".

Los datos que obtuvo en modelos de ratón comprobaron que el ajo contiene una serie de compuestos bioactivos que proporcionan un blindaje a las neuronas, haciéndolas más resistentes ante agentes tóxicos que están presentes en patologías neurodegenerativas. Es por ello que, a largo plazo, este estudio "apunta a desarrollar un nutracéutico, porque hemos visto que este extracto tiene efectos preventivos en la Enfermedad de Alzheimer", señaló la bióloga UdeC.

Los resultados de esta investigación, que por su impacto ya fue destacada en varios medios de comunicación; están contenidos en la tesis titulada "Efectos neuroprotectores del extracto de ajo negro chilote sobre la toxicidad del péptido beta amiloide", que Javiera Gavilán desarrolla en el marco del Magíster en Neurobiología impartido por la Facultad de Ciencias Biológicas de la UdeC.

Ella es parte de la primera generación de estudiantes que ingresó a este programa de postgrado, el cual también ha impulsado líneas de investigación en torno a enfermedades neurodegenerativas como Parkinson y Creutzfeld Jacob, además de estudios sobre receptores de glicina y niveles fisiológicos de nutrientes, entre otros.

"Cuando este magíster se abrió fue como una luz, fue súper bueno porque era específicamente lo que yo quería aprender y lo que me hacía falta también en mi carrera como bióloga", señaló Gavilán.

Este es, precisamente, el principal enfoque del Magíster en Neurobiología que imparte la Facultad de Ciencias Biológicas de la UdeC, programa que apunta a formar científicos especialistas en neurobiología, con una base teórica y práctica sólida en estudios relacionados al sistema nervioso y con una fuerte orientación hacia lo experimental, donde se encuentra el sello distintivo de este programa. Este se fundamenta en la calidad de los grupos de investigación vinculados al claustro académico del programa, en la disponibilidad de equipamiento de primera línea para la investigación de frontera en neurobiología y en la vigencia de proyectos con financiamiento externo.

Enfoque hacia lo experimental

El Magister en Neurobiología de la FCB busca formar profesionales especializados en la comprensión científica profunda de problemáticas neurobiológicas relevantes en nuestra sociedad actual, como por ejemplo, enfermedades neurodegenerativas, adicción, dolor crónico, desórdenes alimenticios, y depresión, entre otros.

Gracias a este enfoque, el programa ha desarrollado líneas de investigación en enfermedades neurodegenerativas como Alzheimer, Parkinson y Creutzfeld Jacob; compuestos naturales, además de estudios sobre receptores de glicina y niveles fisiológicos de nutrientes, entre otros.

Uno de los creadores del programa fue el doctor Leonardo Guzmán, quien comentó que este "pretende ser un magíster con parámetros modernos, con mucho énfasis en generar nuevo conocimiento a través de los proyectos de tesis, por lo que está pensado para quienes estén motivados en aprender técnicas, protocolos, procedimientos de experimentación en neurobiología celular y molecular y comportamiento animal"

El académico detalló que este programa está orientado a "estudiantes que hayan tenido una base de fisiología y bioquímica más o menos importante, por lo tanto, alumnos de Bioquímica, de Bioingeniería, Química y Farmacia, Biología, Biología Marina; también para las carreras del área de la salud, como Kinesiología, Medicina, Tecnología Médica, Obstetricia, Odontología", entre otras.

A través de su programa académico, que considera asignaturas básicas, de especialidad, complementarias y seminarios; el alumno egresado será especialista en neurobiología experimental y contará con competencias científicas que permitan el inicio de una carrera asociada a la investigación y a la divulgación de la disciplina. Además, tendrá la capacidad de comunicar, integrar y analizar críticamente y generar nuevos conocimientos en el área de la neurobiología en un contexto bioético.

Mayor información:

Paula Veloso Aguilera
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MAGÍSTER
EN NEUROBIOLOGÍA

PRIMER INGRESO AÑO 2018

★ **3** Alumnos regulares

★ **14** Profesores acreditados

Universidad de Concepción

FACULTAD DE CIENCIAS BIOLÓGICAS
Universidad de Concepción

DESCRIPCIÓN

Nuestro programa se fundamenta en la necesidad de formar profesionales especializados en la comprensión científica de problemáticas neurobiológicas relevantes en nuestra sociedad, como, por ejemplo, enfermedades neurodegenerativas, adicción, desórdenes alimentarios, entre otras. De manera convergente a esta creciente necesidad, nuestra Facultad cuenta con grupos de investigación en neurobiología de alta visibilidad internacional, los cuales desarrollan sus proyectos de investigación con financiamiento externo y equipamiento de primera línea. El sello distintivo de nuestro programa es la formación de especialistas capaces de desarrollar neurobiología experimental a través de una visión integrativa de elementos moleculares, celulares y neurofisiológicos, en un marco bioético.

PLAN DE ESTUDIOS

Durante el primer año, el plan de estudios del programa está conformado por cuatro asignaturas básicas y una asignatura de especialización. Junto con ellas, se deberá realizar la defensa del proyecto de tesis. Durante el segundo año se deberá ejecutar y presentar la tesis de grado, en la cual se desarrollarán temáticas de neurobiología experimental en los laboratorios de investigación de nuestra Facultad.

LINEAS DE INVESTIGACIÓN

- Aspectos Celulares y Moleculares de la Neurodegeneración
- Bases Celulares y Moleculares de la Función Neuronal y Glial
- Neurofarmacología Molecular y Celular

MALLA CURRICULAR

1er Semestre

Fisiología del Sistema Nervioso (5 créditos)
Neurociencia Avanzada I (3 créditos)
Formulación de Proyecto (1 créditos)
Elección de una asignatura de especialización (2 créditos)

2do Semestre

Experimentación Básica en Neurobiología (7 créditos)
Proyecto de Tesis

3er Semestre

Tesis

4to Semestre

Tesis

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www.csbiol.udec.cl/postgrado/neurobiologia
postgradocsbioludec
postcsbioludec

La formación de doctores: Nuevas tendencias y oportunidades de internacionalización

Concepción Peiró Vallejo
Profesora Titular, Departamento de Farmacología, Facultad de Medicina
Coordinadora del Programa de Doctorado en Farmacología y Fisiología
Subdirectora de la Escuela de Doctorado (EDUAM)
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Significado de los estudios de Doctorado

El título de Doctor es el máximo grado académico que puede otorgar la institución universitaria. En Europa, tras el proceso de armonización del Espacio Europeo de Educación Superior (EEES), los estudios universitarios se ordenan en los niveles sucesivos de Grado, Máster y Doctorado, coincidiendo con la mayoría de las instituciones académicas a nivel global. La finalidad primordial de los estudios de Doctorado es la formación de profesionales de la investigación, que representan piezas clave de una sociedad basada en el conocimiento, como es la actual. Por otra parte, no hay que olvidar que el término "doctor" deriva del verbo latín "docere", es decir, enseñar. No en vano el título de Doctor es un requisito imprescindible para el desarrollo completo de una carrera académica universitaria.

Educación doctoral: más allá de la tesis doctoral

Durante muchos años, el eje central de los estudios de Doctorado ha sido la realización de la tesis doctoral, consistente en un trabajo de investigación original desarrollado por el candidato a recibir el título de Doctor. Hoy en día, la tesis doctoral sigue siendo sin duda el elemento principal de los estudios de Doctorado, si bien se hace cada vez más hincapié en una formación más amplia e integral del doctorando que le permita adquirir una serie de competencias y habilidades, relacionadas con la investigación, pero también con otros aspectos más transversales y de utilidad más allá de la carrera investigadora (Figura 1).

Efectivamente, uno de los objetivos actuales de los programas de doctorado es poner a disposición de sus estudiantes actividades formativas que les permitan manejar mejor aspectos generales directamente asociados con la labor investigadora, como cursos de conducta responsable en investigación, de análisis de datos, de manejo de bibliografía, de escritura de trabajos científicos o de proyectos de investigación, o de pensamiento crítico, entre otros.

Pero más allá de los aspectos específicos relacionados con la actividad puramente investigadora, se busca también hoy en día proveer a los estudiantes de doctorado con una serie de capacidades de carácter transversal e intersectorial, que les puedan ser útiles en áreas que no sean las puramente académicas o investigadoras. Hablamos, por ejemplo, de habilidades de comunicación oral y escrita, de habilidades interpersonales, de manejo de conflictos y negociación, de desarrollo de carrera profesional (Figura 1). En efecto, hay que tener en cuenta que cada vez más instituciones no académicas y empresas demandan doctores, que puedan ejercer de líderes de su sector. Por otra parte, es una realidad que en la actualidad un gran número de los doctores egresados ya no permanecen en la academia, por lo que conviene dotarles de este tipo de herramientas profesionales que puedan serles de utilidad tanto dentro como fuera de la institución universitaria.

Internacionalización y movilidad en el Doctorado, ¿cuáles son sus ventajas?

Uno de los principales aspectos transversales que se busca desarrollar hoy en día durante los estudios de Doctorado es la internacionalización y la movilidad de estudiantes. Pero, ¿qué ventajas puede ofrecer al candidato a Doctor el realizar una estancia de investigación en el extranjero que puedan compensarle del esfuerzo personal, logístico y económico que supone ese desplazamiento? Si el grupo receptor y el momento de realización de la estancia se eligen bien, sin duda las ventajas son múltiples y abarcan diferentes planos de interés.

En primer lugar, la movilidad puede, y debería, suponer una expansión del horizonte técnico y científico del estudiante, con oportunidades para intercambiar conceptos, ideas y experiencias en entornos diferentes del ha-

"La educación doctoral hoy en día tiene como meta, junto con la realización de la tesis doctoral, ayudar a que el futuro Doctor adquiera una serie de conocimientos y habilidades, relacionadas o no con la investigación, que le proporcionen una preparación más completa e integral de cara al mundo profesional".

bitual. Esto además favorece la creación de redes de contactos, lo que en inglés se conoce como "networking", que pueden ser de gran utilidad para la empleabilidad y el desarrollo de la carrera profesional futura del candidato a Doctor.

Por otra parte, la movilidad puede suponer un claro beneficio en cuanto a competencias idiomáticas y de comunicación, especialmente si la estancia se realiza en países que no comparten la lengua materna del estudiante de Doctorado. En cualquier caso, e independientemente del idioma hablado, el estudiante tendrá que desarrollar más que nunca su capacidad organizativa y sus habilidades de comunicación y de relaciones interpersonales, al salir de su zona de confort en el centro de origen.

Por otra parte y más allá del beneficio profesional, durante la movilidad el estudiante toma consciencia de otros usos y costumbres, de otros ámbitos culturales y amplía su círculo relacional, lo que puede contribuir muy positivamente a su capacidad de adaptación y crecimiento personal. De hecho, la superación de los retos encontrados durante la estancia de movilidad puede también fomentar y afianzar la confianza en sí mismo del candidato a Doctor.

Por último, no olvidemos que, más allá del beneficio para los propios estudiantes de Doctorado, la movilidad puede resultar altamente provechosa para los propios grupos de investigación e instituciones implicados, que pueden beneficiarse mutuamente de su respectiva visión investigadora y educativa. De hecho, no cabe duda de que la movilidad de los estudiantes de Doctorado se verá claramente favorecida si se fomentan acuerdos y alianzas formales entre instituciones académicas investigadoras de diferentes países, ya sea a nivel global de la propia institución, ó de manera más particular entre los programas de Doctorado y grupos de investigación implicados. En este sentido, no sólo la Universidad como institución, sino también otras agencias de investigación y financiación, adquieren un rol de responsabili-

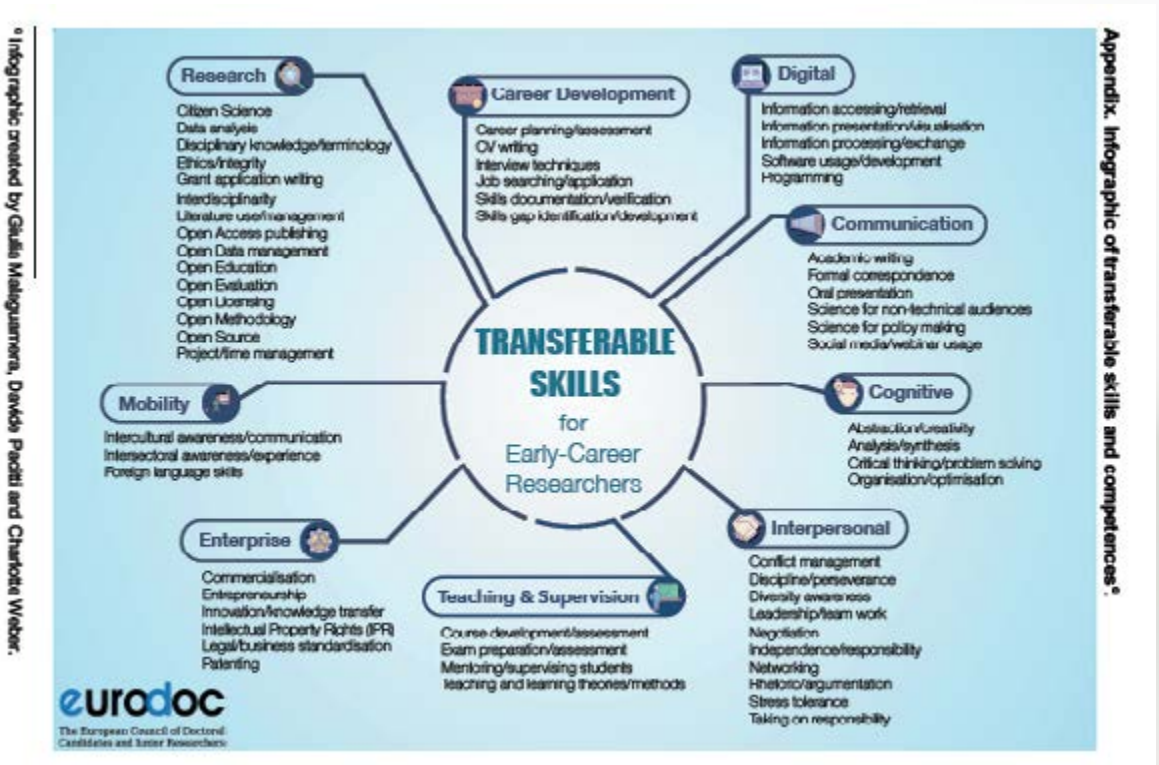

Figura 1. Esquema gráfico de habilidades y competencias transferibles de interés para la mejor empleabilidad y competitividad de slos estudiantes de Doctorado. Fuente: "The European Council for Doctoral Candidates and Junior Researchers (EURODOC)". http://eurodoc.net/skills-report-2018.pdf

dad en la facilitación de la movilidad y en la supresión de barreras económicas y logísticas que permitan a los doctorandos desarrollar una experiencia internacional beneficiosa para su desarrollo profesional y personal.

Conclusión y perspectivas

En resumen, la educación doctoral hoy en día tiene como meta, junto con la realización de la tesis doctoral, ayudar a que el futuro Doctor adquiera una serie de conocimientos y habilidades, relacionadas o no con la investigación, que le proporcionen una preparación más completa e integral de cara al mundo profesional y que fomenten su empleabilidad en un mercado cada vez más competitivo. Es por tanto una obligación y un reto actual de los Programas de Doctorado y de los agentes responsables de la educación doctoral velar por facilitar, en la medida de posible, la mejor formación investigadora y transversal, para que los egresados puedan constituirse en profesionales de referencia que contribuyan al avance de sus respectivos sectores.

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Efectos del ozono médico sobre la Peroxidación Lipídica y la actividad de la Aldehído Deshidrogenasa 2 cerebralen ratas alcohólicas en abstinencia

María Teresa Díaz-Soto1* (Dra. Farmacéuticas, Profesora Auxiliar), Ángela Fraga Pérez (MC Toxicología), Jacqueline Dranguet Vaillant1 (MC Farmacología), Erik Jerez (Lic. Cs. Farmacéuticas), María de los Angeles Bécquer (MC Farmacología), Mayté Casanova (MC Toxicología), Maikel Arteaga Cruz (MC Farmacología), Olga Sonia León Fernández (Dra. Ciencias Farmacológicas, Profesora Consultante),

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RESUMEN:
El consumo crónico de etanol provoca aumento en la acumulación de acetaldehído y formación de Especies Reactivas de Oxígeno, lo que provoca daño a nivel cerebral, esto se prolonga durante el estado de abstinencia. Actualmente el tratamiento utilizado para contrarrestar las afectaciones provocadas por el alcoholismo y que se manifiestan durante la abstinencia alcohólica es extremadamente limitado.
Objetivos: Este estudio se propuso determinar los efectos protectores del Post-condicionamiento Oxidativo con Ozono sobre la actividad de la enzima Aldehído Deshidrogenasa 2 cerebral y las concentraciones de malonildialdehído sistémica y cerebral después de 2 semanas de abstinencia alcohólica en ratas Lewis machos
Método: La administración de etanol por vía oral fue "ad libitum" .Las ratas se distribuyeron en cuatro grupos: (I) Control, recibió agua durante todo el experimento, (II) Etanol (ET-OH), (III) Etanol + Ozono, como el grupo II pero después de la abstinencia alcohólica fueron sometidas a postcondicionamiento oxidativo con ozono a concentración de 20 µg/mL, dosis de 1 mg/kg, 15 tratamientos por l insuflación rectal y (IV) Etanol + Oxígeno, como el grupo III pero tratado con oxígeno (26 mg/kg). . Se les fue introduciendo gradualmente el consumo de etanol en soluciones de 10, 20, 30, y 40 % (56 días). Sesenta ml de agua o solución de etanol fueron colocados en cada caja diariamente por 8 semanas. Cada 24 horas fueron medidos los volúmenes de etanol o agua consumidos. Fue evaluada la tolerancia farmacológica. Se determinó las concentraciones en suero de MDA antes y después de la aplicación de ozono Finalmente los cerebros fueron removidos para estudiar el estado redox mediante Aldehído deshidrogenasa 2 mitocondrial y malonildialdehído.
Resultados: Fueron significativos los efectos del ozono sobre los niveles de MDA. La aplicación de Ozono restableció los niveles de MDA y la actividad dela ALDH2 mitocondrial, enzima que transforma el Acetaldehído en acetato inactivo
Conclusiones: El tratamiento con Ozono disminuyó el estrés oxidativo sistémico durante la abstinencia alcohólica a nivel de Sistema Nervioso Central. Estos resultados demuestran el papel que juega el Ozono como protector del daño a este nivel en la abstinencia alcohólica. Estos resultados demostraron que el ozono ejerció un efecto protector contra el daño oxidativo en el cerebro, preservando funciones importantes del Sistema Nervioso Central(SNC)
Abstract: Ethanol withdrawal (EW) increases acetaldehyde accumulation and reactive

"El consumo crónico de etanol puede provocar daño mitocondrial lo que conlleva a disfunción de la ALDH y por tanto aumento de las concentraciones de acetaldehído al cual se le ha adjudicado un papel importante en el daño cerebral característico del alcoholismo y del Síndrome de Abstinencia Alcohólica"

oxygen species formation that promote damage to the brain. It is necessary to emphasize that while EtOH abuse and dependence are widespread, treatment options are extremely limited.

Purpose: This study aimed at investigating the protective effects of Ozone Oxidative Postconditioning (Ozone OxPost) on mitochondrial aldehyde dehydrogenase 2 (ALDH2) and MDA concentrations induced by oxidative stress after 2 weeks of EW in rats.

Method: Oral "ad libitum" administration of ethanol was used. Rats were divided into 4 groups. The groups were (I) Control, received tap water during the entire duration of the experiment, (II) Ethanol (ET-OH), (III) Ethanol + Ozone, as group II, but at the beginning of EW, rats were submitted to Ozone OxPost at a concentration of 20 µg/mL, dose 1 mg/kg, 15 treatments by rectal insufflation was used and (IV) Ethanol + Oxygen, as group III but rather than ozone, rats were treated with oxygen (26 mg/kg).The rats were gradually introduced to ethanol consumption through ET-OH solutions from 10, 20, 30, and 40% (56 days). The determination systemic concentrations of MDA was before and after the application
of ozone. Afterwards, brains were promptly removed for oxidative stress studies. (Mitochondrial aldehyde dehydrogenase 2 (ALDH2) and MDA concentrations)

Results: Of special note were ozone´s effects on MDA levels. Ozone therapy re-established MDA and mitochondrial aldehyde dehydrogenase 2 (ALDH2), the same enzyme that transforms acetaldehyde into inactive acetate.

Conclusions: Ozone treatment improved the systemic oxidative stress during EW. These results demonstrated that Ozone protected the brain against oxidative injury, improving important functions of the Central Nervous Systems (CNS).

Keywords Ozone, Ethanol Withdrawal, Oxidative Stress, Central Nervous System

Introducción

El alcoholismo es una enfermedad crónica caracterizada por la ingesta compulsiva y excesiva de bebidas alcohólicas a un nivel que interfiere con la salud física y mental, al igual que con las responsabilidades sociales, familiares, económicos o laborales. El alcoholismo es un tipo de drogadicción, en la cual hay tanto dependencia física como mental (1, 2). Produce una fuerte dependencia al consumo de alcohol etílico, manifestándose el Síndrome de Abstinencia Alcohólica cuando no es posible su ingesta (3).

El alcoholismo es incompatible con la vida (4), existen dos factores que afectan de forma significativa la calidad de vida del paciente alcohólico: Por una parte, los efectos directos del etanol sobre diferentes sistemas de neurotransmisión a nivel de Sistema Nervioso Central (SNC) como son la transmisión GABAérgica, dopaminérgica y la glutamatérgica, al provocar desequilibrio neuronal y con ello alteraciones conductuales que se prolongan durante el Síndrome de Abstinencia Alcohólica (SAA). Otro factor que afecta la calidad de vida del paciente alcohólico está relacionado con el metabolismo del etanol, que ocurre por vías enzimáticas y vías no enzimáticas. Las enzimas que metabolizan al etanol son la Citocromo P4502E1 que se localiza en los microsomas, la Catalasa localizada en peroxisoma y la Alcohol Deshidrogenasa citosólica, el metabolito formado es el acetaldehído que es degradado por la enzima Aldehído Deshidrogenasa mitocondrial (ALDH2) (5). El consumo crónico de etanol puede provocar daño mitocondrial lo que conlleva a disfunción de la ALDH y por tanto aumento de las concentraciones de acetaldehído al cual se le ha adjudicado un papel importante en el daño cerebral característico del alcoholismo y del Síndrome de Abstinencia Alcohólica (6, 7, 8). Por otra parte, las vías no enzimáticas por las que se metaboliza el etanol provocan la formación de Especies Reactivas de Oxigeno favoreciéndose la Peroxidación Lipídica (POL) generándose malonildialdehído (MDA) (cuyo metabolismo también ocurre por la ALDH). Se ha evidenciado la formación de aductos entre el acetaldehído y el MDA. Ambos pueden provocar afectaciones en el Sistema Nervioso tanto Autónomo como Central (9)

Numerosos estudios han demostrado que la aplicación del Ozono médico constituye una conducta beneficiosa para muchas enfermedades degenerativas y afecciones orgánicas (Diabetes Mellitus, Artritis Reumatoide, hernia discal, presencia de convulsiones). Trabajos experimentales y clínicos confirman sus acciones terapéuticas. En estudios realizados en modelos de hepatotoxicidad por CCl_4 y en modelos de isquemia/reperfusión hepática, se han evidenciado los efectos pleiotrópicos del ozono, el cual ha restablecido el balance redox y preservado la integridad mitocondrial lo que se relaciona con la protección de la actividad de la enzima Superóxido Dismutasa a nivel hepático.

A partir de los objetivos de este trabajo se realizó un estudio después de dos semanas de abstinencia alcohólica sobre la influencia del ozono sobre el metabolismo del etanol y la ocurrencia de peroxidación lipídica a nivel cerebral mediante la determinación de la actividad cerebral de la enzima Aldehído deshidrogenasa mitocondrial y las concentraciones de MDA respectivamente.

"A los grupos que fueron tratados con ozono se le administró por insuflación rectal 1mg/kg, es decir, 5 cc durante 14 días de abstinencia. Los grupos que fueron tratados con oxígeno se le administraron 5 cc por insuflación rectal durante los 14 días de la abstinencia"

Materiales y Métodos

Se utilizaron 20 ratas Lewis (250-300g) provenientes del Centro Nacional de Producción de Animales de Laboratorio de (CENPALAB; Mayabeque, Cuba). Las ratas fueron alimentadas con alimento proveniente del Centro Nacional para la Producción de Animales de Laboratorio (CENPALAB; Mayabeque, Cuba) y agua/etanol "ad libitum" (45 g y 60 mL) en cajas hogar bajo ciclos de luz y oscuridad de 12 horas a una temperatura de 20+2°C. Para medir el peso y el consumo de alimentos se empleó una balanza analítica (Denver Instrument XP-3000). Todos los procedimientos con los animales se realizaron según lo establecido por la unidad de gestión de la calidad (UGC) del Centro de Investigaciones y Ensayos Biológicos del Instituto de Farmacia y Alimentos de la Universidad de La Habana en concordancia con la regulación de la Unión Europea para el tratamiento de animales de experimentación (Bethesda, MD, USA)(10)
El estudio fue dividido en diferentes etapas las cuales se muestran a continuación:

A- Etapa administración de etanol (Inducción del alcoholismo)
Etapa cuyo objetivo es inducir y estimular el desarrollo de conductas reforzadoras a través del consumo exploratorio, recompensa y adicción al etanol. Se desarrolla con una escalada de consumo in crescendo para generar dependencia física al etanol (11).

B- Etapa Tolerancia Farmacológica
Etapa cuyo objetivo es la determinación de conductas compulsivas al consumo del etanol propias de los alcohólicos. La preferencia de mayores concentraciones del etanol (entre 10% y 40% durante 2 semanas) permite establecer el grado de tolerabilidad y adaptación en el SNC.

C- Etapa de Abstinencia
Etapa cuyo objetivo es analizar los resultados de la deprivación del consumo de etanol característicos de los alcohólicos en Abstinencia Alcohólica. Al grupo tratado con etanol se le retiró abruptamente el suministro de etanol durante 2 semanas. El comportamiento de los grupos experimentales en esta etapa demuestra la necesidad o no que presenten de consumir etanol lo cual es evaluado mediante test conductuales.

Determinación del consumo de líquido

Se estableció dos grupos iniciales: Grupo Control de 5 ratas y Grupo tratado con etanol de 15 ratas. El Grupo tratado con alcohol se le administró dosis ascendentes cada 14 días de experimentación (5% día 1, 10% día 15, 20% día 29, 30% día 43 y 40% día 57). Los animales tenían acceso solamente a la botella con solución de etanol. A partir del día 70 hasta el día 84 el grupo tratado con etanol se sometió a una etapa de Tolerancia Farmacológica suministrándole "ad libitum" etanol a las dos concentraciones (10% y 40%) para evaluar el biomodelo de alcoholismo desarrollado. Se realizó control del peso corporal, consumo de comida y líquido durante las distintas etapas de inducción y tolerancia al consumo crónico del etanol (84 días) y al final de los 14 días siguientes de Abstinencia Alcohólica.

Distribución de los grupos experimentales

Al terminar el día 84 del experimento el grupo tratado con etanol fue dividido en 3 grupos de 5 ratas formando un total de 4 grupos de ratas con 5 ratas cada uno:
Grupo 1: AGUA
Grupo 2: ETANOL
Grupo 3: ETANOL+OXÍGENO
Grupo 4: ETANOL+OZONO

Aplicación de la Ozonoterapia y Oxígeno.

El Ozono (O3/O2) se generó mediante el OZOMED (equipo producido por el Centro Nacional de Investigaciones Clínicas de Cuba, CNIC) a partir del oxígeno clínico, el cual constituyó alrededor del 3% de la mezcla O3/O2 + O2. A los grupos que fueron tratados con ozono se le administró por insuflación rectal 1mg/kg, es decir, 5 cc durante 14 días de abstinencia. Los grupos que fueron tratados con oxígeno se le administraron 5 cc por insuflación rectal durante los 14 días de la abstinencia.

Obtención del Suero

Las ratas de estudio fueron sedadas en una campana de cristal con éter dietílico. Después de haber corroborado el efecto, se procedió a la extracción de 5ml de sangre por el plexo ocular. La sangre extraída se depositó en via-

les para centrifugar, debidamente rotulados y se mantuvo en reposo a temperatura ambiente durante 10 min para la retracción del coágulo. Posteriormente el coágulo fue separado cuidadosamente de las paredes del vial con un capilar y se centrifugó a 3 600 rmin-1 durante 15 min para la obtención del suero. El suero obtenido se congeló a -20˚C para luego ser empleado en una serie de determinaciones bioquímicas. La extracción de sangre se realizó en 3 etapas: al inicio (día 0), después de consumir el 20% de alcohol (día 29) y al finalizar la abstinencia (día 98) (12).

Preparación de Homogenado de cerebro.

Los homogenados se obtuvieron en el día 99 del experimento empleando el homogenizador de cuchillas (Edmund Bühler HO4, Alemania) para lo cual se utilizó 2 g de tejido en 20 ml de buffer sucrosa-tris-HCl pH=7,4 0,2M según se describe en el PNO/TEC/0303. Posteriormente se centrifugó 10 min, 3000 rpm a 4oC (Sigma Centrifuge 2K15, Reino Unido). El sobrenadante se guardó para la obtención de las fracciones subcelulares y para la determinación de los marcadores de estrés oxidativo (13)

Determinación de Malonildialdehído (MDA)

El MDA se midió como indicador de la POL utilizando el método colorimétrico que emplea el 1-metil-2-fenil indol como cromógeno reportado por Esterbauer, H en 1990. La condensación de una molécula de MDA con dos moléculas de 1-metil-2-fenil indol, bajo condiciones de acidez da como resultado la formación de un cromóforo con una absorbancia máxima de 586 nm. Una solución de 7,6 mM de 1-metil-2-fenil indol en metanol al 33% en acetonitrilo se preparó inmediatamente antes de ser utilizada. Una alícuota de 325 µL de este reactivo se hizo reaccionar con 200 µL de muestra se agitó y se añadió 75 µL de ácido clorhídrico 37 %. Tras una nueva agitación de microtubos de ensayo se incubaron 40 min a 45 ºC. Posteriormente, cuando alcanzaron la temperatura ambiente, se centrifugó a 3000 rpm durante 15 min y se leyó la absorbancia a 586nm contra blanco reactivo. Se utilizó una curva patrón de 1,1,3,3-tetraetoxipropano para calcular las concentraciones finales (14).

Determinación de la actividad de ALDH

Fraccionamiento celular. Obtención de mitocondria.

Para la obtención de la fracción mitocondrial de células cerebrales en la medición de la actividad de ALDH, el homogenado se centrifugó a 900 g por 15 minutos obteniéndose el pellet. El pellet obtenido de este paso se hizo resuspender con 10 ml de buffer sucrosa-tris-HCl pH=7,4 0,2M. Posteriormente se rehomogenizó a 340 rpm y se centrifugó a 9000g por 10 minutos. El sobrenadante obtenido se volvió a centrifugar a 104.000 g por 60 min y el precipitado mitocondrial se lavó dos veces y se re suspendió en 10 ml de la solución tampón, que se utilizó para la determinación de la ALDH mitocondrial de cerebro. Se comprobó la pureza de la fracción mitocondrial aislada mediante la determinación de la enzima Alcohol Deshidrogenasa. La enzima alcohol deshidrogenasa fue utilizada, como enzima marcadora de la fracción microsomal. Menos del 5% de la actividad alcohol deshidrogenasa se encontró en la fracción microsomal, indicando una contaminación mínima de enzimas citosólicas (15).

Determinación de proteínas totales

La concentración de proteínas totales en el homogenado de cerebro fue determinada por el método descrito por Bradford (1976). Este método se basa en la coloración de las proteínas presentes en el medio con el reactivo azul de Coomassie (Sigma), detectable a 595 nm. La cuantificación se llevó a cabo mediante una curva de calibración con albúmina sérica bovina (BSA) (Sigma) como patrón de referencia (16).

Determinación de aldehído deshidrogenasa cerebral.

La actividad de la ALDH2 se midió por el aumento de la producción de NADH a 340 nm. La mezcla de reacción contenía: 60 mM de tampón de fosfato de sodio (pH 8,5), 1 mM de NAD+ + EDTA 1 mM y las proteínas mitocondriales (0,5 mg / ensayo). La reacción se mantuvo durante 2 min a temperatura ambiente, la reacción enzimática se inició mediante la adición del sustrato (10 mM propionil aldehído). El cambio de absorbancia se controló durante 30 segundos, 1, 2, 3 y 4 minutos para calcular la tasa de producción de NADH. La actividad específica de ALDH2 se calculó uti-

"Los resultados de la etapa de tolerancia farmacológica en la que se expone al animal a dos concentraciones de etanol, una baja 10% y otra alta 40%, para que consuma la que prefiera se observó que hubo un incremento significativo ($p < 0.05$) en el consumo de la solución etílica más concentrada lo cual indica la preferencia de las ratas en estudio por el etanol".

Figura 1

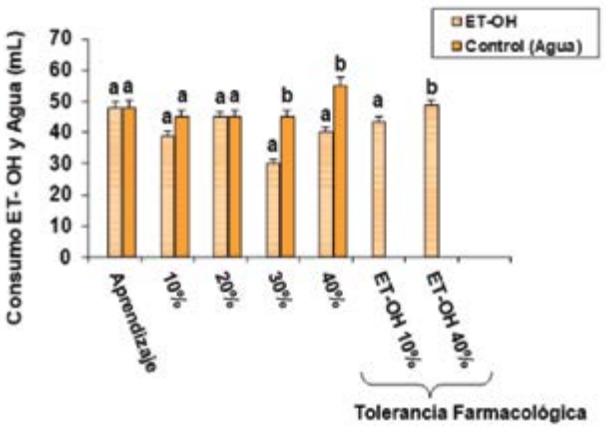

Figura 1. Esquema gráfico de habilidades y competencias transferibles de interés para la mejor empleabilidad y competitividad de slos estudiantes de Doctorado. Fuente: "The European Council for Doctoral Candidates and Junior Researchers (EURODOC)". http://eurodoc.net/skills-report-2018.pdf

"La implementación de un modelo de alcoholismo, que mimetice la sintomatología del humano alcohólico, es un requisito indispensable para el estudio de los efectos terapéuticos de fármacos candidatos a ser utilizados en el tratamiento del alcoholismo, principalmente en la etapa de la "sufrida" abstinencia".

lizando el coeficiente de extinción molar de la reducción de NAD+ de 6,22 × 106 cm2 a 340 nm (Merck Index) y 1 unidad representa una reducción de NAD+ (+ 1 nmol/min/mg de proteína) a temperatura ambiente (17,18).

Procesamiento estadístico

Los datos experimentales obtenidos fueron sometidos a un análisis exploratorio donde se detectaron puntos aberrantes (outiers). Además se estimaron parámetros descriptivos como media y desviación estándar.

Para determinar diferencias entre grupos en una variable en cuestión se utilizó un ANOVA de clasificación simple, seguida de un ensayo de comparación de medias (Student-Newman-Keuls) y el test de Student. El nivel de significación estadístico empleado en todos los casos fue como mínimo de p< 0.05. Para las correlaciones lineales se empleó el coeficiente de correlación de Pearson. Los datos fueron procesados utilizando en paquete estadístico: STATISTICA versión 6.0 para WINDOWS.

Resultados

La Figura 1 muestra el consumo de líquido en los animales objeto de estudio durante las diferentes etapas del desarrollo del modelo, el grupo control que consumió agua (Control) y el grupo que ingirió etanol a diferentes concentraciones. A todas las concentraciones que les fue suministrado el etanol los animales en estudio consumieron la solución alcohólica, no existieron diferencias significativas ($p<0.05$) en el volumen de solución etanólica (10%-40%) consumido durante la inducción del alcoholismo (56 días). Los resultados de la etapa de tolerancia farmacológica en la que se expone al animal a dos concentraciones de etanol, una baja 10% y otra alta 40%, para que consuma la que prefiera se observó que hubo un incremento significativo ($p < 0.05$) en el consumo de la solución etílica más concentrada lo cual indica la preferencia de las ratas en estudio por el etanol. En la etapa de Abstinencia a las ratas alcoholizadas se les retiró el etanol y durante 2 semanas se registró la ingesta de agua. Como puede apreciarse los animales alcoholizados consumieron más agua ($p < 0.05$) que los controles indicando las afecta-

Tabla 1

Grupos experimentales	Actividad de Aldehído Deshidrogenasa mitocondrial en cerebro (mM/mL/min de NADH)
Control (Agua) 6	.33 ± 0.021(a)
Etanol 1	.23 ± 0.050(b)
Etanol + Ozono 4	.85 ± 0.430(c)
Etanol + Oxígeno 0	.97 ± 0.031(b)

Tabla I: Actividad de la Aldehído Deshidrogenasa 2 en homogenado de cerebro de las ratas estudiadas.

ciones metabólicas, entre otras manifestaciones, asociadas a la avidez por el agua durante la abstinencia.

La Figura 2 representa las concentraciones de malonildialdehído (MDA) a nivel sistémico en los diferentes grupos objeto de estudio, antes y después de la aplicación del Ozono. El grupo que consumió etanol mostró elevadas concentraciones de MDA respecto a los restantes grupos, mientras que el tratado con ozono manifestó disminución de las concentraciones de MDA con respecto a la etapa previa a la administración de Ozono.

En la Tabla I se presenta la actividad de la ALDH2 cerebral en los grupos estudiados, manifestándose aumento de la actividad de la enzima en los grupos ozonizados con respecto a los no ozonizados que consumieron etanol.

La Figura 3 representa las concentraciones de MDA a nivel cerebral, en el grupo que fue ozonizado existió un comportamiento en las concentraciones del producto de la peroxidación lipídica similar al grupo control mientras que en los restantes grupos las concentraciones de MDA fueron significativamente mayores

Discusión

El alcoholismo es una de las toxicomanías que se manifiesta con más frecuencia a nivel mundial. El paciente alcohólico pasa por diferentes etapas desde la adaptación al consumo del alcohol hasta llegar al llamado punto de "no retorno" (19). La implementación de un modelo de alcoholismo, que mimetice la sintomatología del humano alcohólico, es un requisito indispensable para el estudio de los efectos terapéuticos de fármacos candidatos a ser utilizados en el tratamiento del alcoholismo, principalmente en la etapa de la "sufrida" abstinencia. El modelo seleccionado en nuestro trabajo tuvo en cuenta su acercamiento a las prácticas del alcohólico de ahí que se empleó el consumo de alcohol "ad libitum" ya que es el más semejante al alcoholismo en humanos (20). El consumo de etanol a las diferentes concentraciones (Figura 1) demostró la tendencia de los grupos experimentales a adquirir la conducta adictiva, lo cual se corroboró con los resultados de la Prueba de Tolerancia Farmacológica, al manifestarse la preferencia por la solución etanólica de mayor concentración. Estos resultados se corresponden con lo planteado por otros autores al afirmar que "El consumo crónico genera un progresivo requerimiento de dosis mayores de sustancia para conseguir un efecto similar. La tolerancia puede considerarse un intento del organismo para retornar a la homeostasis, esto es, a las condiciones anteriores al consumo" (21, 22). Este modelo experimental permite no solo profundizar en los mecanismos de acción terapéutica, del agente en cuestión, sino que hace posible ampliar los conocimientos relacionados con la etiopatogenia alcohólica. Por otra parte, los hallazgos científicos que fundamentan los efectos terapéuticos del ozono, por un mecanismo denominado Pre/Post condicionamiento oxidativo, constituye-

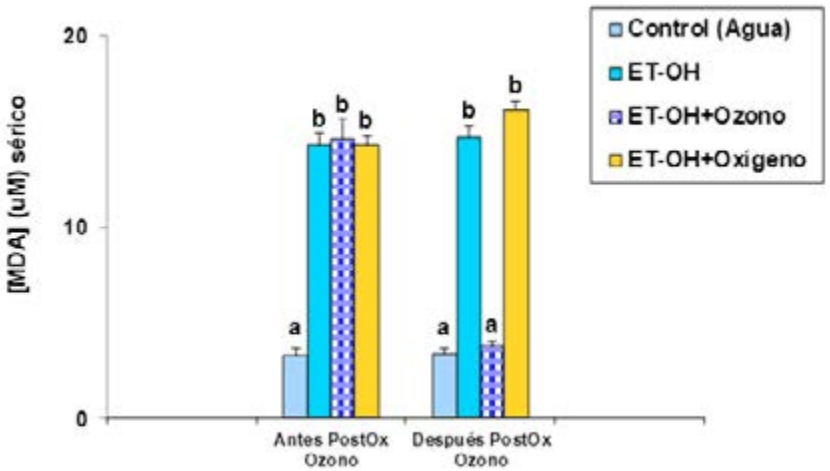

Figura 2

Figura 2. Concentración de MDA séricos en los grupos experimentales estudiados. Antes del PostOx Ozono corresponde al final de la etapa de Tolerancia, antes de la abstinencia y antes del PostOx. Después del PostOx Ozono representa el final del tratamiento con ozono, el final de la Abstinencia y el punto final del estudio experimental. Se representan la media ± EEM. Letras diferentes indican diferencias significativas (p<0.05), para cada grupo experimental, antes y después del PostOx Ozono*

Figura 3

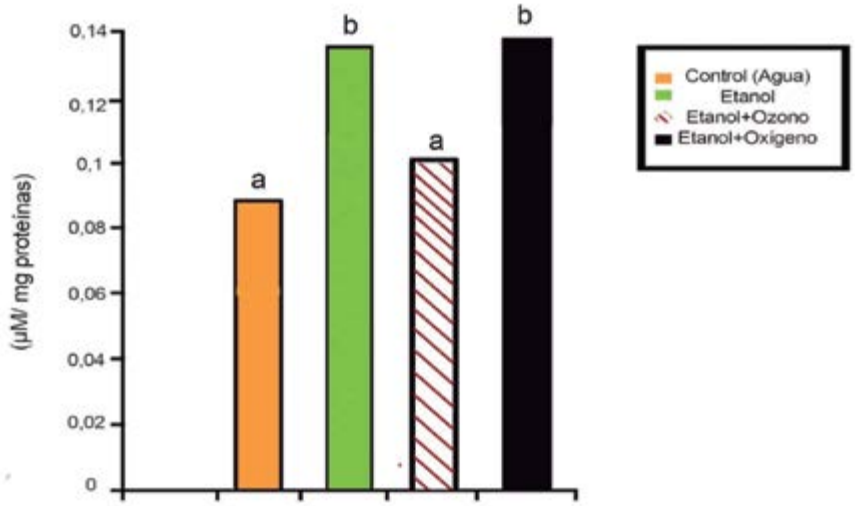

Figura 3. Concentraciones de MDA, en homogenado de cerebro, en las diferentes condiciones experimentales. Las concentraciones de MDA en cerebro de rata, corresponde con el final de la Abstinencia, del PostOx y del experimento (105 días) Se representan la Media ± EEM. Letras diferentes indican diferencias significativas (p<0.05).*

ron la base científica que justifica abordar el estudio de los efectos del ozono, sobre la sobreproducción de ERO y las alteraciones de algunas de las funciones básicas del SNC que se modifican o alteran en el sujeto alcohólico. Los cambios fisiopatológicos en la función celular, inducidos por la exposición crónica de alcohol, se derivan del propio metabolismo del etanol que incluye la generación de acetaldehído, radicales libres (como el hidroxietil) y NADH, entre otros causando Estrés Oxidativo. Es así como mucha de la sintomatología asociada a las disfunciones orgánicas y conductuales del alcohólico son el resultado del daño que promueven estas moléculas oxidadas, sobre el hígado y el Sistema Nervioso Central, tanto durante la tolerancia como en la abstinencia. (23)

El acetaldehído, derivado del metabolismo del etanol y de reconocida toxicidad, es transformado a ácido acético por la enzima Aldehído Deshidrogenasa mitocondrial, isoforma 2 (ALDH2). Esta enzima metaboliza eficientemente el acetaldehído, producido durante el metabolismo del etanol, pero también metaboliza otros aldehídos lipídicos tóxicos como el MDA y 4-hidroxi-2-nonenal, entre otros (24). El consumo crónico de etanol induce lesiones en los complejos I y III de la cadena de transporte electrónico mitocondrial, con afectaciones en la transferencia de electrones desde el flavín-mononucleótido del complejo I al complejo III, se produce acumulación del complejo flavín-mononucleótido-semiquinona y una generación incrementada de radicales superóxido dentro del complejo I. Estas lesiones, inducidas por el consumo crónico de etanol incrementan la producción de superóxido y peróxido de hidrógeno dentro de la mitocondria afectando a la integridad mitocondrial y con ello la función de la enzima ALDH2 (25).

El consumo de etanol es un detonante muy importante para la generación de estrés oxidativo- nitrosativo en el hepatocito y ya sea a corto o a largo plazo, puede desencadenar la muerte de la célula, el tejido y el organismo en general. Algunos factores que están involucrados en la generación de estrés oxidativo-nitrosativo por etanol son: cambios en el estado redox intracelular como resultado del metabolismo oxidativo del etanol, hipoxia celular, porque las alteraciones en el metabolismo redox afectan el consumo de oxígeno y la producción de ATP en la mitocondria.

El metabolismo del etanol y el estrés oxidativo asociado, producen una serie de compuestos de alta reactividad, capaces de unirse a un gran número de residuos proteicos. El resultado de estas uniones es la generación de nuevas entidades bioquímicas que, de forma genérica, reciben el nombre de aductos.

Estos aductos, según su composición, pueden clasificarse en cuatro grandes grupos: aductos derivados del acetaldehído, que provienen de la formación de enlaces entre el acetaldehído y residuos lisina o grupos amino de distintas proteínas. Estos enlaces pueden ser covalentes o no covalentes, lo que determina la estabilidad del aducto formado, algunos productos aldehídicos derivados de la peroxidación lipídica, como el MDA o el 4-hidroxinonenal,

también son capaces de formar aductos con proteínas, también se ha documentado la formación de aductos mixtos o híbridos, que son aquellos formados por la unión de acetaldehído y MDA a un mismo sustrato, finalmente, en presencia de átomos de hierro, los radicales hidroxietilos formados en la oxidación del etanol, pueden formar aductos con proteínas. La unión de grupos aldehído a una biomolécula resulta en una alteración estructural y consecuentemente, funcional de dichos sustratos. Así, se ha descrito que tras su unión con moléculas de acetaldehído (formación de aductos), la actividad de algunas enzimas se reduce o incluso se suprime. El metabolismo del etanol y la consecuente formación de aductos en el SNC, están implicados en los efectos tóxicos del etanol en otros órganos y tejidos y en la generación de alteraciones en el cerebro.

Los aductos de acetaldehído pueden formar complejos con serotonina llamados tetrahidrocarbolinas, ambos complejos aumentan la preferencia por etanol y pueden inhibir competitivamente la MAO y la COMT.

En el presente estudio existió un incremento en las concentraciones de MDA en los grupos no tratados con ozono lo cual indica peroxidación lipídica. Este aldehído tiene una especial importancia en el alcoholismo y en la abstinencia alcohólica ya que el MDA es un sustrato de la Aldehído Deshidrogenasa (ALDH2). Esta enzima es responsable del metabolismo del acetaldehído (inductor del daño cerebral por consumo crónico de etanol) y otros aldehídos lipídicos producidos por el metabolismo del etanol. Por lo tanto el incremento del MDA sugiere la inhibición de ALDH2, la cual puede ser consecuencia de las especies reactivas de oxígeno y nitrógeno (26). La administración de ozono preservó la actividad de la enzima ALDH2 cerebral y disminuyó las concentraciones de MDA, séricas y cerebrales.

Estos resultados indican el efecto protector del ozono sobre el daño que provocó el etanol y por tanto la capacidad de estimular la recuperación funcional de una de las enzimas que de forma más significativa participa en su metabolismo. Al tener en cuenta que la propia ALDH2 participa en el metabolismo del MDA, la disminución de las concentraciones del mismo indican que también el ozono fue capaz de disminuir el proceso de POL. Estos resultados se corresponden con estudios anteriores (27) donde se evidencia, tanto preclínica como clínicamente, el efecto antioxidante del ozono y sus consecuencias favorables sobre trastornos conductuales durante la abstinencia alcohólica.

El mecanismo por el cual el ozono médico provoca efectos antioxidantes se resumen en dos moléculas intermediarias: Peróxido de Hidrógeno y los productos de POL (MDA). El O_3/O_2 induce la formación de H_2O_2 e hidroperóxidos, en cantidades pequeñas, los cuales son capaces de actuar sobre los eritrocitos, plaquetas, monocitos y células endoteliales estimulando la acción de la Glutatión Peroxidasa (GSH-Px) para formar H_2O. Esta actividad prooxidante estimula no sólo la GSH-Px sino también la Glutatión Reductasa (GSH-Rx) y aumenta los niveles de Glucosa 6-Fosfato (G-6P). Como consecuencia se produce un aumento del intercambio iónico, de los niveles de 2,3 difosfoglicerato (2,3-DPG), de los niveles de ATP y, por ende, de la glicólisis. El O_3/O_2 también estimula un aumento de la relación NAD+/NADH activando la vía del monofosfato de hexosa dependiente induciendo la liberación de ATP de los eritrocitos y vasodilatación (28).

Se ha demostrado que el ozono es capaz de proteger a las células hepáticas frente al daño producido por los radicales libres del oxígeno; así como la preservación de las concentraciones de glucógeno hepático, evitando la sobreproducción de lactato, que en el homogenado de hígado está asociado al daño hepatotóxico (29). El restablecimiento en las concentraciones de MDA, sugiere la preservación de la enzima ALDH2, evitando la acumulación de acetaldehído, a nivel sistémico y del SNC, con la consiguiente disminución en la formación de aductos.

La disminución de las concentraciones de MDA y el aumento de la actividad de la enzima ALDH2 sugieren que el tratamiento con ozono protegió la integridad mitocondrial manifestada con la disminución de la peroxidación lipídica lo cual puede ser con secuencia del incremento en el metabolismo tanto del acetaldehído como del malonildialdehido sustratos de la enzima y relacionados con el daño cerebral presente en la Abstinencia Alcohólica.

La aplicación de nuevos procedimientos experimentales, así como la identificación de posibles biomarcadores que sean confiables y sensibles, es un gran avance que puede contribuir a implantar un adecuado sistema de prevención y diagnóstico para detectar, en lo posible, el daño ocasionado por la ingestión de etanol.

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"Estos resultados se corresponden con estudios anteriores (27) donde se evidencia, tanto preclínica como clínicamente, el efecto antioxidante del ozono y sus consecuencias favorables sobre trastornos conductuales durante la abstinencia alcohólica".

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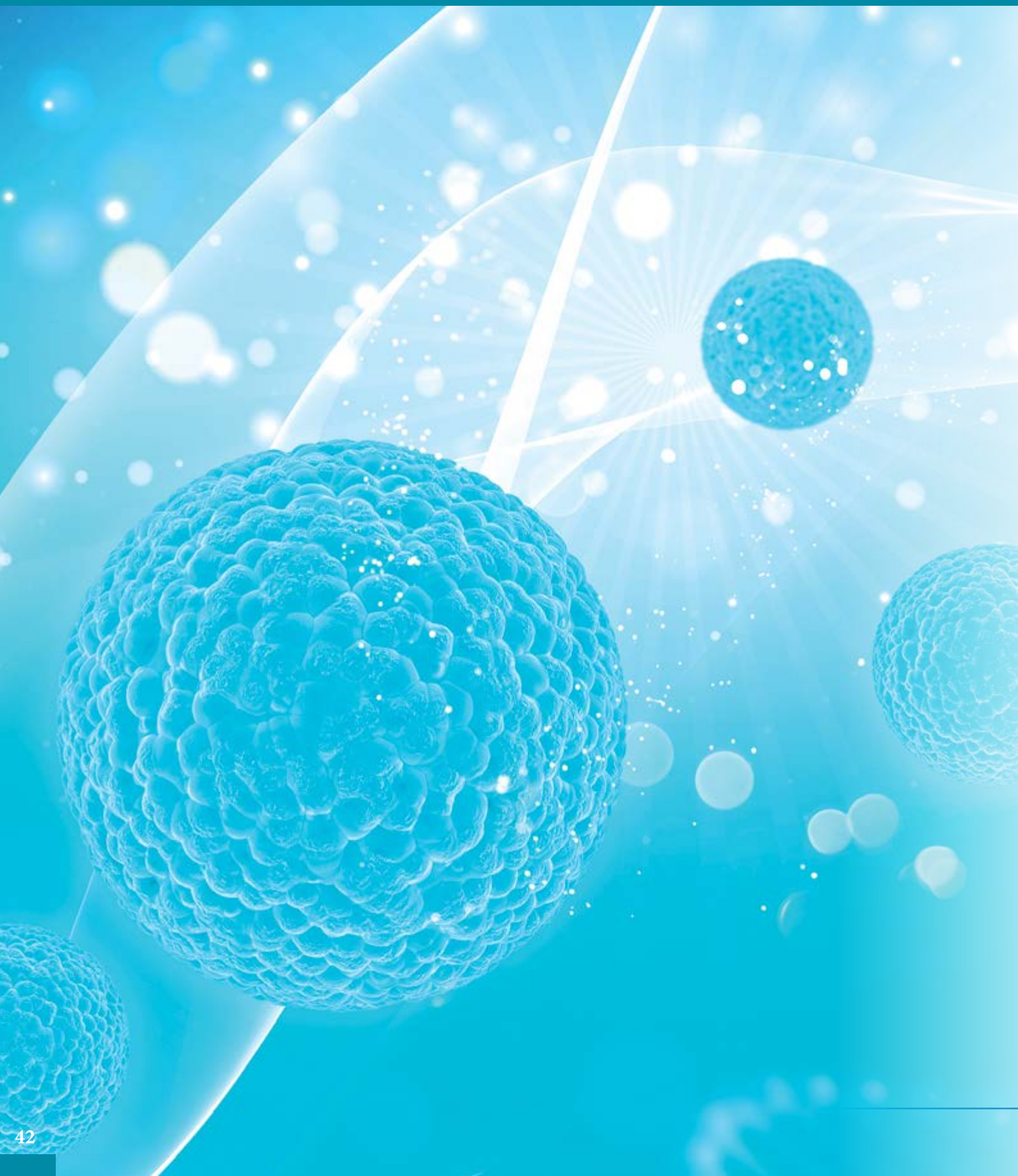

Función fisiológica y fisiopatológica de las membranas asociadas a mitocondrias (MAMs): Posible rol en el procesamiento de APP y en la disfunción mitocondrial en la enfermedad de Alzheimer.

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Resumen

La Enfermedad de Alzheimer (EA) es la principal forma de demencia que afecta a los adultos mayores. Se caracteriza por una muerte neuronal progresiva, especialmente en hipocampo y corteza, y uno de los principales eventos celulares con los que cursa esta patología es la disfunción mitocondrial. Se postula que esta enfermedad neurodegenerativa se produce principalmente por el aumento en los niveles del péptido beta-amiloide (Aβ), que se genera a partir del procesamiento proteolítico de la proteína precursora amiloide (APP). Se ha descrito que tanto APP como la maquinaría enzimática de la vía amiloidogénica y no amiloidogénica presentaría diferente localización espacial; mientras que los componentes de la via no amiloidogénica se encontrarían en la membrana plasmática y compartimentos secretores tardíos, los compartimentos endosómales, Golgi y estructuras tipo balsas lipídicas denominadas MAMs (del inglés Mitochondria-Associated RE Membranes) serían los encargados de procesar APP por la vía amiloidogénica, y producir Aβ. Las MAMs son dominios especializados entre Retículo endoplásmico (RE) y mitocondrial, donde existe una alta aposición entre ambos organelos de entre 10-20 nm, que les permite comunicarse entre sí tanto física como bioquímicamente, lo que facilita las funciones de esta región tales como la homeostasis del calcio, colesterol, fosfolípidos, y el metabolismo mitocondrial. Existe evidencia reciente que ha encontrado que las funciones localizadas en las MAMs estarían aumentadas significativamente en los modelos animales y muestras de pacientes con la EA. En esta revisión bibliográfica estudiaremos los principales roles fisiológicos y fisiopatológicos de las MAMs, y su impacto en la funcionalidad mitocondrial y en el procesamiento de APP.

Introducción

La Enfermedad de Alzheimer (EA) es una enfermedad neurodegenerativa, caracterizada por la pérdida progresiva de la memoria y aprendizaje (Castellani et al., 2010). Si bien se desconoce su causa, histológicamente se han identificado dos marcadores; las placas seniles y los ovillos neurofibrilares (Ballard et al., 2011). El péptido Aβ se postula como principal agente responsable de la EA (Hardy and Higgins, 1992), por su capacidad de generar dishomeostasis del Ca^{+2}, ATP, y lípidos (Blass et al., 2000; Lal et al., 2007)electrophysiological, and neuroimaging examinations. Therefore, impairment in energy metabolism in AD can not be attributed to loss of brain substance or to electrophysiological abnormalities. Among the characteristic abnormalities in the AD brain are deficiencies in several enzyme complexes which participate in the mitochondrial oxidation of substrates to yield energy. There include the pyruvate dehydrogenase complex (PDHC. En este contexto, la mitocondria es un factor clave, ya que funciona como tampón de Ca^{+2}, productor de ATP, y participa en el metabolismo de los lípidos, por ende, se ha asociado la disfunción mitocondrial como evento temprano de la pérdida de la conexión de la red neuronal, falla sináptica, y finalmente de la muerte neuronal (Onyango et al., 2016).

La producción del péptido Aβ comienza por el procesamiento proteolítico de la proteína precursora amiloide (APP), proteína integral de membrana que se puede procesar por dos vías. La vía no amiloidogénica involucra la participación de α-secretasa, una enzima que pertenece a la familia de metaloproteasas ADAM (del inglés A Disintegrin and Metalloprotease), y γ-secretasa, complejo compuesto por 4 proteínas: Presenilina 1 o 2 (PS1 o PS2), Nicastrina (Nct), Aph-1 (del iglés Anterior Pharinx-Defective-1) y Pen-2 (del inglés presenilin enhancer 2); de estas proteínas, PS1 y PS2 tienen la actividad catalítica y el resto tiene funciones regulatorias. Estas enzimas forman los productos: APPsα (APP soluble α), C83(APP-C83), AICD(Dominio intracelular carboxi terminal de la proteína APP) , y p3. Por otra parte, en la vía amiloidogénica, actúan β y γ-secretasa, que forman APPsβ, C99 (APP-C83), AICD y el péptido Aβ (Muller et al., 2017; Suh and Checler, 2002)the cause and progression of both familial and sporadic AD have not been fully elucidated. The autosomal-dominant inherited forms of early-onset Alzheimer's disease are caused by muta-

tions in the genes encoding APP, presenilin-1 (chromosome 14.

Se ha descrito que tanto APP como la maquinaría enzimática de ambas vías presentarían diferente distribución subcelular; la vía no amiloidogénica se produciría principalmente en la membrana plasmática y compartimentos secretorios tardíos, mientras que la vía amiloidogénica se produciría principalmente en compartimentos endosomales, balsas lipídicas (Zhang and Song, 2013) y estructuras denominadas MAMs (Membranas de Retículo Endoplásmico asociadas a mitocondrias) (Area-Gomez et al., 2012). Por lo tanto, sería en estos últimos compartimentos intracelulares donde se produciría el péptido Aβ, y no en la superficie de la membrana plasmática, como se ha sugerido clásicamente.

Las MAMs son dominios especializados entre retículo endoplásmico (RE) y mitocondrial, de una naturaleza semejante a las balsas lipídicas ya que son microdominios ricos en esfingomielina y colesterol resistentes a los detergentes (Aria Gómez et al., 2012), donde existe una asociación estrecha entre ambos organelos (10-20 nm), que les permite comunicarse entre sí física y bioquímicamente, facilitando las funciones de esta región tales como metabolismo del Ca^{+2}, lípidos, y regular funciones mitocondriales (Bernard-Marissal et al., 2018). Existe evidencia reciente de que las funciones asociadas a las MAMs aumentan significativamente en los modelos animales de la EA y en las células de pacientes con EA (Area-Gomez et al., 2012). Es por ello por lo que a continuación describiremos los principales roles que cumplen los MAMs y sus implicancias en la EA cuando estos se incrementan de forma patológica.

Rol de los MAMs en la transferencia de Ca^{+2}

Los MAMs tiene un rol en la transferencia de Ca^{+2} desde RE a la mitocondria, donde participarían los proteínas IP3R (receptor de IP3), Sigma R1 (Receptor intracelular no opioide sigma 1) a nivel de RE, y VDAC1(canal selectivo de aniones dependiente de voltaje 1) a nivel de la membrana mitocondrial externa (MME). Esta comunicación inter-organelar coordina tanto el metabolismo de Ca^{+2} como preservación de la integridad de la MME (Vance, 2014). Los estudios de proteómica para evaluar los niveles las proteínas del complejo IP3R-VDAC1, mostraron un aumento dependiente de la edad de los niveles de proteína VDAC1 en el hipocampo de ratones Tg2576, un modelo de la EA (Hsiao et al., 1996), comparadas con ratones control de la misma edad (6-12 meses). Además, en tejido cerebral de pacientes con EA (etapa Braak V-VI) los niveles de VDAC1 aumentaron significativamente (55%) comparados con individuos control (Cuadrado-Tejedor et al., 2011).

En relación con el IP3R, existen trabajos que muestran resultados distintos dependiendo de los modelos de estudio. En células CHO-7PA2 que expresan APPV717F, se encontró un incremento en los niveles del IP3R en comparación a células control, mismo resultado que se observó en modelos celulares de sobreexpresión de APP swedish (APPswe); sin embargo, en muestras de corteza de ratones transgénicos Tg2576 la expresión de IP3R no se vio afectada significativamente (Oules et al., 2012).

Por otra parte, en ratones P301L tau, que fueron inyectados en la amígdala con el péptido $A\beta_{1-42}$, no se observaron cambios significativos en muestras de corteza para la proteína GRP75 en comparación a muestras de corteza control. Esto también fue observado por análisis de western blot de la corteza temporal de individuos con EA, donde no se observaron cambios significativos en los niveles de GRP75 en comparación con individuos control (David et al., 2006).

Además, Dyzma describió una evidencia funcional de que el incremento en las asociaciones MAMs estaría relacionada con una mayor transferencia de Ca^{+2} desde el retículo hacia la mitocondria mediado por la vía IP3R-VDAC1 en un modelo de la EA. En células SHSY5Y al co-incubar con una agonista de la chaperona del IP3R Sigma R1 (Receptor Receptor intracelular no-opioide sigma 1), localizada en la interfaz RE-mitocondria, y el péptido Aβ, se producía una potenciación del flujo de Ca^{+2} desde el RE hacia la mitocondria (Dyzma et al., 2012).

Rol de los MAMs en mitofagia

El proceso de mitofagia es un proceso degradativo que regula el control de calidad mitocondrial, en el cual las mitocondrias depolarizadas asociadas un fenotipo hiperfisionado activan su reciclaje selectivo, y por lo tanto la célula es capaz de adaptar la cantidad y calidad de las mitocondrias dependiendo de sus necesidades energéticas. Este proceso estaría mediado por receptores Atg8 y Atg32 (Proteína relacionada con la autofagia 8 y 32), que reclutan la maquinaria autofágica en la mitocondria; evento que promueve su envoltura por un fagóforo que posteriormente se fusiona con un lisosoma (Bockler and Westermann, 2014).

De acuerdo al trabajo de Gelmetti (Gelmetti

et al., 2017), se logró describir que la formación de autofagosomas (mitofagosomas) ocurriría en las MAMs; y que este proceso estaba mediado por la relocalización de las proteínas mitofágicas PINK1, BECN1 en las MAMs. En otro trabajo, Böckler en un modelo de levaduras realizó mutantes de proteínas MAMs Mmm1, Mdm10, Mdm34, Mdm12 encargadas de estabilizar el acoplamiento RE-mitocondria, observado que estas mutantes eran defectuosas específicamente para el proceso mitofágico. Además, encontró una asociación entre atg8 y Mmm1, resultado que indicaría que la asociación MAM es necesaria para iniciar el proceso de la mitofagia de forma selectiva (Bockler and Westermann, 2014).

Rol de los MAMs en transporte mitocondrial

La comunicación entre el cuerpo celular y los terminales sinápticos en las neuronas se basan en el transporte axonal, el cual es altamente eficiente para las mitocondrias, ya que deben alcanzar sitios especializados con alta energía de en un breve tiempo. Se ha descrito una correlación positiva entre el transporte de mitocondrias axonal con proteínas MAMs, en donde sería necesaria la interacción de Mfn2 (mitofusina 2), MIRO1/2 (Rho GTPase 1 y 2 mitocondrial) y las proteínas motoras quinesinas y dineinas para promover el transporte de las mitocondrias retrogrado y anterógrado, entre el soma neuronal y el axón (Bernard-Marissal et al., 2018).

Sin embargo, existe escasa información del papel de las MAMs en el compartimento axonal, y si la disfunción de MAM puede afectar específicamente el transporte axonal, ya que la mayoría de los estudios están hechos en líneas celulares. En modelos de esclerosis lateral amiotrófica (ELA) y de neuropatías hereditarias motoras y sensoriales Villegas en un explante de nervio usando mutantes de proteínas MAMs SigmaR1 y Mfn2, logró visualizar que había una degradación axonal asociada a una falla en el transporte mitocondrial, lo que estaría sugiriendo que este transporte es dependiente de la formación de las MAMs (Villegas et al., 2014).

En motoneuronas, al suprimir la expresión de la proteína SigmaR1 disminuyó el transporte anterógrado mitocondrial, conduciendo a una acumulación mitocondrial en la zona distal del axón, interesantemente esta acumulación también condujo a un cambio en la morfología mitocondrial (Bernard-Marissal et al., 2018).

Rol de los MAMs en dinámica mitocondrial

La dinámica mitocondrial es el proceso que regula la fisión y fusión de mitocondrias, dependiendo de los requerimientos energéticos de la célula, suceso donde participan pro-

teínas para fisión como DRP1 y Fis1, y para fusión, como Mfn1,2 y OPA1. Se ha descrito que estos complejos MAMs se encontrarían directamente asociados con un rol directo en la dinámica mitocondrial, ya que se ha encontrado que tanto DRP1 como mitofusina 1 y 2 se encontrarían reclutados en estos complejos, sin embargo, se le han adjudicado a los MAMs un rol en la desarrollo un fenotipo más fisionado. De hecho, cuando hay una disrupción de los complejos MAMs por la deleción de Sigmar1, se observó una alteración en la dinámica mitocondrial, que condujo a que las mitocondrias presentasen un fenotipo mitocondrial más fusionado (Bernard-Marissal et al., 2018).

Otra proteína altamente estudiada que coordina la fusión mitocondrial es Mfn2, proteína que forma homodiméros entre RE y mitocondria (ya que se encuentra expresada en ambos organelos), o heterodímeros con Mfn1 (se encuentra sólo en mitocondria). En efecto, en células MEFs (del inglés Mouse Embryonic Fibroblasts) de un ratón MFN2-KO, hubo una menor obtención de MAMs por fraccionamiento subcelular, lo que estaba directamente correlacionado con la evidencia funcional que mostró que había una menor liberación de Ca^{+2} del RE a la mitocodnria, comparados con MEFs WT (Filadi et al., 2018). Sin embargo, en el año 2012 el grupo de Orci (Cosson et al., 2012), en el mismo modelo, se observó un efecto opuesto; por microscopía de transmisión electrónica e inmunofluorescenca en células MEFs del ratón MFN2-KO hubo un incremento en la yuxtaposición de los MAMs (~4.9%), comparadas con MEFs WT (~2,2%), y desde un punto de vista funcional hubo una directa correlación con el aumento de MAMs, ya que había una mayor liberación de Ca^{+2} del RE a la mitocondria en MEFs MFN2-KO que en MEFs WT (Filadi et al., 2018). Interesantemente, estos efectos estuvieron asociados a una disminución de la expresión del MCU (uniportador de Ca^{2+} mitocondrial), el cual media el importe de Ca^{+2} por la membrana mitocondrial interna. Por lo tanto, y a pesar de que aún es controversial el rol de Mfn2 en los MAMs, si podemos establecer que participa activamente como espaciador de la conformación de las asociaciones RE-mitocondria, aunque serán necesarias más investigaciones para abordar este problema.

Rol de los MAMs en la EA

En fibroblastos de pacientes humanos el grupo de Aria Gómez describió que existían distintas conformaciones entre retículo y mitocondria en fibroblastos de pacientes control, y observó que los MAMs principalmente presentaban áreas de superficie de contacto que alcanzaban asociaciones cortas (menores a los 50nm) (superiores al 50%), en tanto que áreas de contacto larga (entre 50 a 200 nm) se encontraban en torno al 30%, y las áreas de contacto muy largas (mayores a los 200nm) sólo alcanzaban entorno al 4%. En contraparte, se observó que en fibroblastos de pacientes con FAD y SAD, tenían áreas de contacto principalmente largas y muy largas, alcanzando porcentajes del 40% y del 35%, respectivamente (Area-Gomez et al., 2012).

Para lograr comprender por qué estas conformaciones altamente especializadas entre retículo y mitocondrias presentan una sobreexpresión, el grupo de Aria Gómez comenzó a estudiar si componentes de la vía amiloidogénica estaban regulando este aumento. Describió que las presinilinas en fibroblastos humanos se encontraban enriquecidas en los MAMs, utilizando marcadores contra proteínas como es FACL-4, proteína descrita clásicamente como proteína MAM. Además, por fraccionamiento subcelular (para separar los MAMs del retículo y mitocondria), se observó que las presenilinas 1 y 2 se encontraban altamente enriquecidas en los compartimentos MAM, respecto de la membrana plasmática, la mitocondria y el retículo endoplasmático. Además, al analizar la actividad de esta proteína por un ensayo enzimático se pudo concluir que son los compartimiento MAMs los que tienen una mayor actividad de la γ-secretasa con respecto a los otros compartimientos subcelulares. En otro trabajo similar, el grupo de Schreiner describió que además este compartimiento MAM era el que presentaba una mayor producción del péptido β-amiloide, respecto de RE, mitocondria y membrana plasmática (Schreiner et al., 2015).

Respecto de la localización de los otros componentes de la vía amilodogénica, se analizó la localización de β-secretasa y APP en compartimentos intracelulares. Primero, por fraccionamiento subcelular con separación de gradiente de sucrosa en fracciones mitocondriales y fracciones endosomales lisosomales, se pudo observar con distintos marcadores para endosomas, Golgi, retículo y MAMs, que APP y BACE1, el componente catalítico de la β-secretasa, cofraccionan parcialmente con marcadores de endosomas tempranos y tardíos, y con marcadores MAM. Sin embargo, faltan evidencias funcionales de la actividad de β-secretasa en estos compartimentos (Pera et al., 2017). Otra observación importante de estos autores fue que en cerebros de ratones WT no había un inmunomarcaje positivo para los fragmentos derivados de la escisión

en el fragmento C-terminal de APP por la β-secretasa (APP-CTF), que corresponden al fragmento C83 y al C99. Esto podría evidenciar 2 fenómenos; el primero es que no estarían presentes en estos complejos MAMs y la otra posibilidad es que es la alta la actividad de la γ-secretasa estaría rápidamente escindido el fragmento APP-CTF de la vía amiloidogéncia (C99) para producir el péptido β-amiloide y el dominio intracelular AICD.
Por ende, utilizando un doble KO para las presinilinas 1 y 2, observaron que los APP-CTF se encuentran cofraccionando con endosomas y con los MAMs. En células COS-7, línea celular de riñón humano, tratadas con DAPT, un inhibidor de la γ-secretasa, se observó una triple colocalización positiva entre C99, SEC61β, una proteína de retículo, y una sonda mitocondrial, indicando la presencia de este fragmento en los MAMs (Pera et al., 2017). Además, en células MEFs del doble KO de las presenilinas utilizando anticuerpos APP-CTF, se observó la presencia de C99 en los MAMs (Pera et al., 2017).
Sin embargo, falta evidencia que indique que efectivamente sería C99 el fragmento que se encuentra en los MAMs, debido a que estos autores utilizaron un anticuerpo que también sería positivo para C83; por lo tanto, faltan más trabajos que indiquen efectivamente es este fragmento de la vía amiloidogénica que estaría localizado en estos compartimientos.
Se observó que los MAMs se encuentran regulados negativamente por las presinilinas. En células MEFs de ratones DKO para las presinilinas, se observó que había un incremento en la colocalización entre marcadores de RE y mitocondria, respecto de células MEFs de ratones WT. Además, al utilizar BI, un inhibidor de β-secretasa, se observó una disminución significativa de la colocalización entre ambas marcas. Por lo tanto, C99 estaría regulando directamente la sobrerregulación MAM. Este resultado también fue confirmado por ensayo de microscopía electrónica, en donde observó que había un incremento en las áreas de contacto largas y muy largas de los MAMs (>200nm) en células DKO, que además presentaban una conformación punteada no superior al 20%, respecto de las células MEFs-WT, que principalmente tenían una conformación punteada (sobre el 80%), en tanto que alcanzaban áreas de contacto en bajo porcentaje (menores del 16%), y las muy largas no más de un 4%. Además, se observó un aumento de la funcionalidad de los MAMs en células MEFs de ratones DKO, respecto de células MEFs de ratones WT (Area-Gomez et al., 2012).
Desde un punto de vista funcional, por ensayos de radioactividad donde se midió la activad de ACAT1, enzima que transforma el colesterol al colesterol éster, se observó que en células WT MEFs tratadas con un inhibidor de la γ-secretasa (DAPT) había un aumento en la actividad de ACAT1 en comparación al vehículo con DMSO y este efecto se revirtió al añadir un inhibidor de la β-secretasa (BI). Cabe destacar que este efecto no se vio revertido en este caso por in inhibidor de la α-secretasa (TAPI); confirmando entonces que sería C99 y no C83 el que estaría regulando las MAMs.
Interesantemente, se observó que había una comunicación entre presinilinas y la proteína Mfn2, ya que ambas participaban en la misma ruta de actividad de las MAMs (Area-Gomez et al., 2012). Para ello, en células WT MEFs comparadas con doble KO (DKO) de las presinilinas 1 y 2 se observó que el escisión de C99 se vio inhibida en células MEFs DKO. Por otra parte, al utilizar un ratón KO para mitofusina 2 en células MEFs y al compararlos con ratones WT MEFs, se observó un efecto similar en células MEFs DKO; ambas presentaban una disminución de escisión de C99. Estos datos establecen entonces que Mfn 2 regularía positivamente el clivaje de C99 y la actividad de γ-secretasa, y, por ende, estaría potenciando la vía amiloidogenica.

Rol de los MAMs en la disfunción mitocondrial

Ha sido ampliamente estudiado que en la EA ocurre una disfunción mitocondrial, pero aún se desconoce la razón de esta alteración. Una de las hipótesis que ha sido ampliamente estudiada por el grupo de Área Gómez (Area-Gomez et al., 2009, 2012; Pera et al., 2017), es que el aumento de la comunicación mitocondrial ER y la función MAM en la EA sería la responsable de esta alteración, y esta alteración esta mediada por una potenciación de la vía amiloidogénica. En efecto, el rol que tendría C99 de la vía amiloidogénica tendría un alto impacto en la funcionalidad mitocondrial. Esta alteración en funcionalidad mitocondrial fue observada mediante ensayos de Seahorse de fracciones enriquecidas en mitocondrias, que permiten medir en tiempo real medir el consumo de oxígeno (ORC), fenómeno asociado directamente a la funcionalidad de los complejos respiratorios de la cadena transportadora de electrones, y por ende, a la capacidad de producir ATP.
Se observó que células MEFs APP-DKO (doble KO de APP) presentaban un mayor consumo de oxígeno en comparación a MEFs WT, indicando que C99 estaría regulando negativamente la función mitocondrial. Este resultado fue confirmado al utilizar un fragmento

En la siguiente esquema se encuentra un modelo propuesto que resume lo expuesto anteriormente en esta revisión:

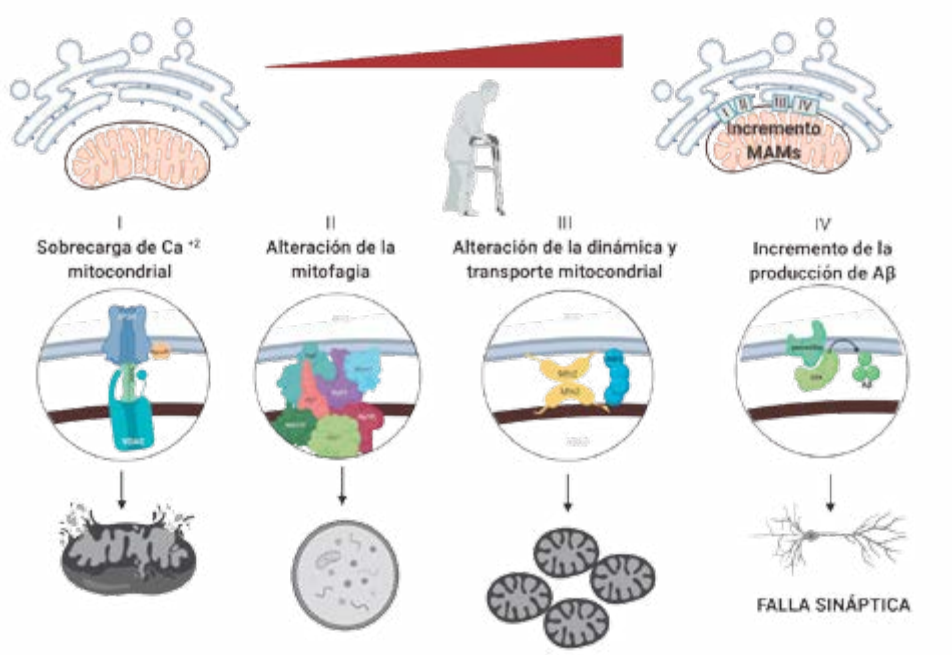

Figura 1. Rol de las MAMs en la EA. Esquema representativo que muestra las vías que estarían involucradas en la falla sináptica. Se puede observar que el incremento en la asociación RE-mitocondria mediaría la disfunción mitocondrial mediante alteración de los componentes encargados de regular la homeostasis de calcio (I), mitofagia (II), transporte y dinámica mitocondria (III), y la producción del péptido Aβ (IV).

de C99 en el ratón APP-DKO donde hubo una disminución significativa de la función mitocondrial en ratones APP-DKO C99, comparadas con ratones APP-DKO. Es más, esta disminución se vio potenciada al utilizar este fragmento C99 en conjunto con un inhibidor de la γ-secretasa (DAPT), indicando que C99 estaría promoviendo la pérdida de la correcta función mitocondrial (Pera et al., 2017).

Por otra parte, en células MEFs DKO (para presinilina 1 y 2), hubo una disminución significativa del consumo de oxígeno respecto a células MEFs WT. Sin embargo, cuando se utilizó un inhibidor de la síntesis de Novo de lípidos (Miriocina), y por ende, bloqueando el flujo de lípidos hacia la mitocondria, hubo una reversión de este fenómeno de disfunción mitocondrial, reflejando en la disminución en la capacidad respiratoria mitocondrial estaría mediada por el aumento del metabolismo lipídico en los MAMs (Pera et al., 2017). Cabe destacar que falta evidencia de posibles efectos de AICD y de los fragmentos de APP soluble β que no se ha descrito hasta el momento, que y pudiesen también estar afectando la funcionalidad mitocondrial. Por lo tanto, podemos concluir que la alteración o mutación las presinilinas impiden el correcto corte de C99, lo que estaría induciendo disfunción mitocondrial, evento que se ha asociado directamente con la falla sináptica y la muerte neuronal en pacientes con EA.

En suma, como hemos visto, es posible que la intercomunicación entre RE y Mitocondrial va más allá de los roles clásicamente atribuidos a estas dos importantes organelas; y permiten pensar que frente a la carencia de selectividad o especificidad de los actuales fármacos indicados en las etapas leves a moderadas de la EA, la identificación de nuevas dianas terapéuticas, que permitan abrir nuevos espacios de desarrollo de moléculas de síntesis, o moléculas biológicas que puedan modular la función de las MAMs, representa una interesante oportunidad para el desarrollo farmacológico de medicamentos neuroprotectores.

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XLI CONGRESO SOCIEDAD DE FARMACOLOGÍA

4 AL 8 DE NOVIEMBRE

Universidad de Concepción

FACULTAD DE CIENCIAS BIOLOGICAS
Universidad de Concepción

Estimadas socias y socios

Sean todas y todos muy bienvenidos a Concepción, la ciudad Universitaria de Chile, que les recibe en un año especial donde la comunidad penquista celebra a la Universidad de Concepción, su emblema más reconocido, en el aniversario de sus primeros 100 años, periodo en el que ha aportado a la generación de conocimiento en las ciencias, las artes y las humanidades, a la política social del país y a su desarrollo.

Hemos trabajado arduamente como directiva para lograr que el presente Congreso cuente con toda la excelencia, experiencia y juventud que caracteriza a nuestra organización. Recibiremos en Concepción a una veintena de investigadores internacionales provenientes desde México, Cuba, Venezuela, Uruguay, Ecuador, Brasil y Argentina, quienes se han interesado en compartir con nosotros sus investigaciones, exposiciones que se sumarán a las de destacados especialistas en farmacología del país y a las de nuestros invitados especiales.

Es así como hemos construido un programa académico y científico de alto nivel que contará con 9 simposios, 2 mini simposios, más de 70 expositores nacionales e internacionales, entre quienes destacan 5 reconocidos conferencistas de prestigio mundial. Las jóvenes huestes científicas también estarán presentes, representados por cerca de 190 investigadores que nos mostrarán sus avances en el desarrollo de sus trabajos de investigación.

Especial mención y agradecimientos debemos por la generosa participación del Instituto-Fundación Teófilo Hernando de la Universidad Autónoma de Madrid, cuyos representantes participarán impartiendo un simposio de sus actividades de I+D del medicamento. También extendemos este reconocimiento a los representantes de La Sociedad Española de Farmacología (SEF), a quienes agradecemos también por haber acogido a una delegación de la Sofarchi en su reciente congreso anual en julio de 2019.

Queridas amigas y amigos, en sus 41 años de existencia la Sofarchi se encuentra en un momento clave de su desarrollo. A consecuencia de las movilizaciones de millones de personas y familias que han ocurrido a lo largo y ancho de nuestro Chile, tenemos el enorme desafío y la responsabilidad de poner nuestra experiencia a disposición de la sociedad, para convertirnos en referentes y líderes de opinión en el área de nuestra experiencia.

Tenemos el deber ético de estar estrechamente vinculados a las necesidades de nuestros ciudadanos. Ya hemos visibilizado este compromiso a través de actividades de extensión, como lo hicimos con las charlas de difusión realizadas en diversos colegios de la comuna de Santa Cruz en el año 2018, las presentaciones en colegios de La Serena a comienzos de este año y nuestra participación en el Brain Awarennes Week Concepción 2019, donde ocho de nuestros socios realizaron exposiciones científicas para más de 2000 alumnos de colegios municipales, un hecho inédito para nuestros registros.

También hemos contribuido a cursos de actualización en temas de Cáncer, Drogas de Abuso y Adicciones, Neurofarmacología y Fármaco-endocrinología, entre otros. Nuestra excelencia científica una vez más se ve reconocida por la revista Frontiers in Pharmacology, que nos ha dedicado una sección especial (Research Topic) donde tenemos cerca de 70 trabajos científicos comprometidos por los socios, de los cuales cerca de 40 se encuentran ya evaluados o en proceso de revisión.

Finalmente quiero agradecer a los todos los miembros de la Directiva, al Editor de la Revista de Farmacología de Chile, Dr. Ramón Sotomayor, al Editor Asociado de Frontiers in Pharmacology, Dr. Gonzalo Yévenes y a todos quienes han dedicado gran trabajo y esfuerzo para lograr los hitos mencionados y permitir el continuo crecimiento de nuestra organización.

Con todos estos elementos, esperamos que este XLI Congreso Anual de la Sociedad de Farmacología de Chile supere con creces sus expectativas científicas y, además, nos permita compartir agradables jornadas de camaradería entre nosotros, disfrutando de las hermosas postales de nuestra histórica ciudad de Concepción.

Un fraterno abrazo

Dr. Jorge Fuentealba Arcos, PhD.
Presidente

PROGRAMA XLI CONGRESO ANUAL SOCIEDAD DE FARMACOLOGIA DE CHILE 2019
UNIVERSIDAD DE CONCEPCION, CONCEPCION, CHILE
LUNES 04 DE NOVIEMBRE

LUNES 04 DE NOVIEMBRE

11:00 – 15:00	INSCRIPCIONES
15:00 – 17:00	INAUGURACIÓN (DR. J. FUENTEALBA) CONFERENCIA 1: DR. A. GARCÍA, ESPAÑA
17:00 – 17:30	COFFEE BREAK
17:30 – 19:30	SIMPOSIO 1: "PURINERGIC SIGNALLING: FROM STRUCTURE-ACTIVITY TO APPLICATION IN PATHOLOGIES"
19:30 – 20:30	CONFERENCIA 2: DR. S. MONCADA, UK
20:30 – 22:00	CÓCTEL BIENVENIDA

MARTES 05 DE NOVIEMBRE

09:00 – 11:00	SIMPOSIO 2: "TOXICOLOGÍA COMO UNA CIENCIA MULTIDISCIPLINARIA"
11:00 – 11:30	COFFEE BREAK
11:30 – 12:30	CONFERENCIA 3: DR. S. STOJILKOVIC, USA
12:30 – 13:30	PREMIO MARDONES (3)
13:30 – 15:00	ALMUERZO LIBRE
15:00 – 17:00	SIMPOSIO 3: "TRANSLATIONAL OPTIONS FOR THE TREATMENT OF DRUG-ABUSE DISORDERS: OPPORTUNITIES, SUCCESSES AND PITFALLS"
17:00 – 17:30	COFFEE BREAK
17:30 – 19:30	MINISIMPOSIOS: "DESREGULACIÓN DEL METABOLISMO PROTEICO EN ESQUIZOFRENIA", "ENVEJECIMIENTO CARDIOVASCULAR"
19:30 – 22:00	SESIÓN DE POSTERS

MIERCOLES 06 DE NOVIEMBRE

09:00 – 11:00	SIMPOSIO 4: "ESTRATEGIAS PARA EL DISEÑO Y DESARROLLO DE NUEVO FÁRMACOS"
11:00 – 11:30	COFFEE BREAK
11:30 – 12:30	CONFERENCIA 4: DR. T. GALLAGHER, UK
12:30 – 13:30	INCORPORACIONES (4)
13:30 – 15:00	ALMUERZO LIBRE
15:00 – 17:00	SIMPOSIO 5: "ENFERMEDADES NEURODEGENERATIVAS Y NEUROPROTECCIÓN"
17:00 – 17:30	COFFEE BREAK
17:30 – 19:30	SIMPOSIO 6: "QUÍMICA MEDICINAL: DISEÑO RACIONAL Y RELACIONES ESTRUCTURA ACTIVIDAD, LA APROXIMACIÓN SINTÉTICA DE NUEVAS SUSTANCIAS BIOLÓGICAMENTE ACTIVAS"
19:30 – 22:00	SESIÓN DE POSTERS

JUEVES 07 DE NOVIEMBRE

09:00 – 11:00	SIMPOSIO 7: "ANTIMICROBIAL ACTIVITY OF HERBAL EXTRACTS AGAINST CLINICALLY RELEVANT PATHOGENS"
11:00 – 11:30	COFFEE BREAK
11:30 – 12:30	CONFERENCIA 5: DR. C. BAUZAT, ARGENTINA
12:30 – 13:30	INCORPORACIONES (2), COMUNICACIONES ORALES (4) (2 CHILE, 2 MÉXICO)
13:30 – 15:00	ALMUERZO LIBRE
15:00 – 22:00	TARDE LIBRE

VIERNES 08 DE NOVIEMBRE

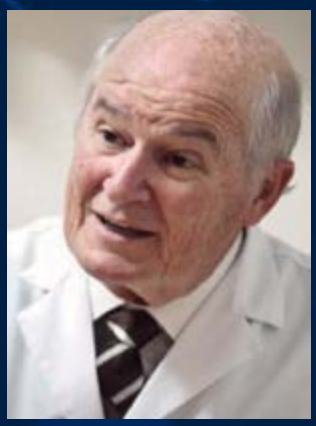
09:00 – 11:00	SIMPOSIO 8: "BIOTECHONOLOGY ASPECTS OF ASPARAGINASE CLINICAL AND INDUSTRIAL DEVELOPMENT"
11:00 – 11:30	COFFEE BREAK
11:30 – 12:30	CONFERENCIA 6: DR. A. BUSH, AUSTRALIA
12:30 – 13:30	COMUNICACIONES ORALES (6)
13:30 – 15:00	ALMUERZO LIBRE
15:00 – 17:00	SIMPOSIO 9: "NEUROBIOLOGICAL TOPICS IN CHRONIC PAIN MANAGEMENT: FROM MOLECULES TO BEHAVIOR"
20:30	CENA CLAUSURA

Conferencistas

Dr. Salvador Moncada, School of Medical Sciences, Manchester Cancer Research Centre, University of Manchester, Inglaterra. Médico, cirujano y farmacólogo hondureño. Realizó labores docentes en las Universidades de El Salvador y Honduras y distintos estudios en los laboratorios Wellcome Research entre 1975 y 1985, cuando estuvo a cargo del equipo de investigación que trabajaba en las prostaglandinas. Su trabajo se ha centrado en efectos farmacológicos de las sustancias vasoactivas, inflamación e interacción entre plaquetas y pared vascular; además de realizar importantes trabajos sobre la trombosis y la arterioesclerosis. Respecto a este último punto, destacó en los años 70 por ser el descubridor de la prostaciclina, un vasodilatador muy potente que actúa como inhibidor de los trombos que obstruyen las arterias.

Fue profesor visitante en diferentes universidades de Europa, Estados Unidos, Hispanoamérica y Japón y desde 1972 ha sido asesor de la Organización Panamericana de la Salud. Sus muchos méritos profesionales le han valido el reconocimiento de todo el mundo; es miembro de la Royal Society, de la Sociedad Británica de Farmacología, de la Sociedad Colombiana de Medicina Interna, de la Sociedad Farmacológica Peruana y académico de Honor de la Real de Medicina de Valencia; es también doctor Honoris causa por las Universidades de Honduras, Cantabria y Complutense de Madrid. Está en posesión de cinco patentes correspondientes a distintos fármacos, y es autor, colaborador o director de unas cuatrocientas publicaciones científicas.

internacionales



Dr. Antonio G. García, Instituto Teófilo Hernando and Departamento de Farmacología, Facultad de Medicina, Universidad Autónoma de Madrid. MD, PhD Presidente de la Fundación Teófilo Hernando (FTH) y Profesor Emérito de Farmacología Universidad Autónoma de Madrid.

Desde 1975 sus estudios se han enfocado en la regulación farmacológica y neuroquímica de los canales iónicos, junto con la señalización del calcio; ambas líneas de investigación proyectadas hacia la comprensión de los principios básicos que gobiernan la liberación exocítica de catecolaminas, la supervivencia neuronal y la muerte neuronal. Más recientemente, se ha orientado al estudio de las enfermedades de Alzheimer (EA) y de Huntington (EH), además de la esclerosis lateral amiotrófica (ELA), a través de la búsqueda de un medicamento neuroprotector capaz de retrasar la progresión de la EA y / o la ELA y mejorar la calidad de vida de los pacientes afectados por estas patologías.

Dr. Ashely Bush, Melbourne Dementia Research Centre, Florey Institute of Neuroscience and Mental Health, The University of Melbourne, Australia.
Psiquiatra y neurocientífico translacional, Director del Centro de Investigación de la Demencia de Melbourne y Jefe de la Unidad de Biología de la Oxidación del Florey Institute of Neuroscience and Mental Health. Es considerado una de las mentes científicas más influentes del mundo, tras descubrir la interacción del beta-amiloide con el zinc como un factor importante en la patogénesis de la enfermedad de Alzheimer y centrarse en la neurobiología de los iones metálicos y del estrés oxidativo en los trastornos neurodegenerativos y psiquiátricos.
Gracias a sus resultados, el estudio de la neurodegeneración se orientó hacia una apreciación de una perturbación subyacente en la homeostasis del metal del cerebro, proporcionando información sobre las enfermedades de Alzheimer, Parkinson y Esclerosis Lateral Amiotrófica. Además, ha desarrollado nuevas pruebas predictivas para la EA y estrategias innovadoras, potencialmente modificadoras de la enfermedad.

Su exitosa carrera ha sido merecedora de diversos galardones, entre ellos el Premio Beeson de la Federación Estadounidense de Investigación sobre el Envejecimiento, el Premio Senator Hatfield de la Asociación de Alzheimer de EE. UU., El Premio Potamkin para la investigación de la enfermedad de Alzheimer, el Premio de Investigación Superior Schering-Plough del Colegio Australiano de Psiquiatras. , una beca de la Federación ARC, el Premio Victoria de Ciencia e Innovación, y actualmente una beca principal de investigación del NHMRC; entre otros.

Es autor de más de 447 publicaciones (más de 41 mil citas), 28 patentes y fundador de 4 empresas de biotecnología.

Dra. Cecilia Bouzat, Instituto de Investigaciones Bioquímicas (INIBIBB), Consejo Nacional de InvestigacioneS Científicas y Técnicas (Conicet), Universidad Nacional del Sur, Argentina.

Doctora en Bioquímica de la Universidad Nacional del Sur (UNS), posteriormente realizó estudios postdoctorales en el Departamento de Fisiología y Biofísica, en la Clínica y Fundación Mayo (Rochester, USA). A partir de su experiencia, la Dra. Bouzat comenzó su carrera como investigadora independiente en el Instituto de Investigaciones Bioquímicas Bahía Blanca (INIBIBB), dependiente de la UNS, a fines de los años 90. En el área de la investigación ha contribuido significativamente al conocimiento a nivel funcional, farmacológico y bioquímico de los canales iónicos activados por acetilcolina y por serotonina del sistema nervioso central, a través de 87 publicaciones científicas, lo que incluye artículos publicados en Nature, Journal of Biological Chemistry y Molecular Pharmacology, entre otras, además de 30 proyectos de investigación financiados por entidades nacionales e internacionales. Producto de su trabajo y esfuerzo, la Dra. Bouzat ha sido distinguida con numerosos premios y distinciones, dentro cuales se destaca el premio L´Oréal-UNESCO for Women in Science, Latin America International Laureate, obtenido el año 2014. El liderazgo de la Dra. Bouzat también se ha reflejado en numerosas participaciones en comités nacionales e internacionales, entre las cuales se destacan la presidencia y vicepresidencia de la Sociedad Argentina de Investigación en Neurociencias (SAN), la membresía titular de la comisión de Ciencias Médicas del CONICET, la membresía del Comité Regional de América Latina de International Brain Research Organization (IBRO-LARC) y la membresía permanente del comité editorial de la revista Molecular Pharmacology. Actualmente, dirige el Laboratorio de Neurofisiología y Farmacología Molecular del INIBIBB y es la directora del INIBIBB, en Bahía Blanca. Además, preside el Comité Regional de América Latina de International Brain Research Organization (IBRO-LARC) y es la directora del programa de Doctorado en Ciencias Farmacéuticas de la UNS.

Dr. Tim Gallagher, PhD Universidad de Liverpool, profesor de Quimica Orgánica de la Escuela de Química de la Universidad de Bristol (Reino Unido),
Sus intereses de investigación abordan la química heterocíclica y abarcan una variedad de temas, los que incluyen el desarrollo de metodología sintética, el estudio del mecanismo de reacción, la síntesis de productos naturales y la aplicación de síntesis a áreas de interés biológico. Tiene intereses particulares en el diseño y síntesis de ligandos nicotínicos selectivos (basados en la citisina) y en la comprensión del mecanismo de acción, así como la química de los sulfamidatos cíclicos como vehículos para la síntesis asimétrica. Tiene colaboraciones dentro del área de carbohidratos basadas en un interés en comprender y explotar las interacciones basadas en carbohidratos.

Su trabajo ha recibido amplios galardones, tales como el Premio Katritzky de la Sociedad Internacional de Química Heterocíclica (1999), el Tilden Lectureship de la Royal Society of Chemistry (RSC) (2004) y el Premio de Química Heterocíclica (2005). Además, es supervisor en el Centro EPSRC de Formación Doctoral en Síntesis Química.

Por otra parte, ha sido consultor y presentador de tres programas de televisión relacionados con la ciencia en el mundo antiguo, entre ellos "Antiguo Descubrimientos III ", serie encargada por History Channel.

XLI CONGRESO ANUAL DE LA SOCIEDAD CHILENA DE FARMACOLOGÍA

¿POR QUÉ PARTICIPAR?

1.- UNIVERSIDAD DE CONCEPCIÓN

CINCO DÍAS DE ACTIVIDADES REALIZADAS EN CONJUNTO CON LA CASA DE ESTUDIOS DEL CENTENARIO. GRACIAS A ELLO, QUIENES PARTICIPAN COMO PATROCINADORES CONTARÁN CON EL RESPALDO TANTO DE SOFARCHI, COMO DE ESTA PRESTIGIOSA UNIVERSIDAD

2.- EXPOSITORES NACIONALES E INTERNACIONALES

PARTICIPACIÓN DE DESTACADOS CIENTÍFICOS PROVENIENTES DE ESTADOS UNIDOS, INGLATERRA, FRANCIA, ESPAÑA Y AUSTRALIA, ENTRE OTROS.

3.- PRENSA

LA SEMANA DE ACTIVIDADES CONTARÁ CON AMPLIA COBERTURA INFORMATIVA Y LA PARTICIPACIÓN DE LOS EXPOSITORES EN MEDIOS DE COMUNICACIÓN.

SOFARCHI
SOCIEDAD DE FARMACOLOGÍA DE CHILE

¿QUIENES SOMOS?

SOFARCHI ES UNA SOCIEDAD CIENTÍFICA SIN FINES DE LUCRO CUYO PRINCIPAL OBJETIVO ES EL FOMENTO DE LA INVESTIGACIÓN EN FARMACOLOGÍA TANTO A NIVEL TEÓRICO, COMO EXPERIMENTAL Y CLÍNICO. DESDE EL PUNTO DE VISTA SOCIAL, NUESTRA SOCIEDAD SE HA ESFORZADO EN PROMOVER EL BUEN USO DE LOS MEDICAMENTOS, DANDO A CONOCER LOS PRINCIPALES EFECTO, TANTO LOS TERAPÉUTICOS COMO LOS ADVERSOS.

XLI CONGRESO ANUAL DE SOFARCHI

CADA AÑO LA SOFARCHI ORGANIZA SU CONGRESO ANUAL, INSTANCIA EN LA QUE PARTICIPAN MÉDICOS, BIOQUÍMICOS, QUÍMICOS FARMACÉUTICOS, BIÓLOGOS, ENTRE OTROS PROFESIONALES; PROVENIENTES DE TODO CHILE Y EL EXTRANJERO.

Universidad de Concepción

CONFERENCES

1. THE EMOTION OF SCIENTIFIC DISCOVERY.

Antonio G. García
Instituto Teófilo Hernando, Departamento de Farmacología y Terapéutica, Facultad de Medicina, Universidad Autónoma de Madrid, Spain

Stephen Hawking said that "science is not only a matter of reason; it is also a matter of romance and passion". This was already written by Santiago Ramón y Cajal in his famous book "Reglas y Consejos sobre Investigación Científica", a hundred years ago. More recently, the National Academy of Sciences of the USA wrote a short guide entitled "ON being a scientist", that summarizes the emotions that a scientist may feel along his carrier when pursuing a problem and finding the response sometimes after years of hard work. I will illustrate the way science is practiced with some experimental findings on the topic of calcium signaling and exocytosis in adrenal chromaffin cells. In doing so, I will focus first on basic science, describing how we arrived to the concept of a functional triad that includes the voltage-gated calcium channels (VGCCs), the endoplasmic reticulum (ER) and mitochondria (MIT). Such triad shapes the cytosolic calcium signals that control both pre-exocytotic and exocytotic responses, the basis of the fight-or-flight stress response of W. Cannon. Then, I will focus on more recent translational research done in chromaffin cells from mouse models of neurodegenerative diseases. I will comment on dysfunctions of Ca2+ and exocytosis occurring even at pre-symptomatic disease stages. I will next make some comments on the failure of clinical trials in AD, to end with some hints on the pressure to "publish-or-perish" and how science is becoming just a mere business for editorials and else.

Recent and ongoing work from AGG'S laboratory is being founded by
1. European Union Horizon 2020 Research and Innovation Programme under the Marie Skoldowska-Curie Grant Agreement 766124);
2. Grant SAF-2016-48892-R, from Ministerio de Ciencia, Innovación y Universidades, Spain; and
3. Fundación Teófilo Hernando, Madrid, Spainen un contexto general y discutiré su importancia con relación a la salud y a la enfermedad.

2. THE PLEASURE OF SCIENCE: MY LIFE IN PHARMACOLOGY.

Salvador Moncada
Manchester Cancer Research Centre, The University of Manchester, U.K.

I will describe the work that opened several fields of investigation. From the mechanism of action of non-steroidal anti-inflammatory drugs, to the discovery of thromboxane synthase and prostacyclin, to the identification of nitride oxide and its metabolic pathway of synthesis. I will finish with a reference of the role of mitochondria in oxidative stress. I will put all this work in a general context and its importance in health and disease will be discussed.

3. CELL TYPE- AND SEX-DEPENDENT TRANSCRIPTOME PROFILES OF RAT ANTERIOR PITUITARY CELLS.

Stanko S. Stojilkovic
Section on Cellular Signaling, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Bethesda, MD, USA

We are investigating receptors and channels expressed in neuronal and endocrine cells and their roles in signaling, gene transcription, and hormone secretion. To gain better understanding of the cell type-specific expression and role of these and other proteins in anterior pituitary cell functions and related disorders, we performed single cell RNA sequencing on freshly dispersed cells from adult male and female rats. Our analysis based on over 7000 cells confirmed the expression of six pituitary-specific cell: folliculostellate cells and hormone producing corticotrophs, gonadotrophs, thyrotrophs, somatotrophs, and lactotrophs. Also identified were endothelial and blood cells from the pituitary capillary network. The expression of numerous developmental and neuroendocrine marker genes in both folliculostellate and hormone producing cells supports that they have a common origin. For several genes, the validity of transcriptome analysis was confirmed by qRT-PCR and single cell immunocytochemistry. Folliculostellate cells exhibit impressive transcriptome diversity, indicating their major roles in production of endogenous ligands and

detoxification enzymes, and organization of extracellular matrix. Transcriptome profiles of hormone producing cells also indicate contributions toward those functions, while also clearly demonstrating their endocrine function. This include the expression of genes encoding numerous voltage-gated, ligand-gated and other channels in hormone-producing but not follicolostellate cell. This survey highlights many novel genetic markers contributing to pituitary cell type identity, sexual dimorphism, and function and points to relationships between hormone producing and folliculostellate cells.

4. DIRECT C-H FUNCTIONALIZATION OF CYTISINE. NICOTINIC RECEPTOR SELECTIVITY AND MECHANISM OF ACTIVATION.

Tim Gallagher
University of Bristol, UK

The talk will cover the application of subtype-selective nicotinic partial agonists to manage nicotine addiction, with a focus on the chemistry of cytisine. Already used for smoking cessation, cytisine (and varenicline) target subsets of nicotinic receptors, and the opportunity to generate novel structural variants of cytisine raises the question of whether more subtype selective ligands are available and of value or indeed are even desirable. The talk will cover recent work in this area, much of which is underpinned by development of C-H functionalisation chemistry that provides very direct and efficient access to new C-10 cytisine derivatives, which in turn, offer more selective subtype profiles. Recent studies (in collaboration with Henry Lester and Dennis Dougherty) have probed the influence of steric vs. electronic factors in determining the binding mode of cytisine. We have also pursued extensive molecular dynamics simulations to probe the mechanism (timing) of signal propagation through the protein scaffold that occurs on ligand association to the receptor, and addressed the question of the applicability and generality of that mechanism across other receptors.

5. GENETIC, PROTEIN AND PHARMACOLOGICAL MODULATION OF HUMAN α7 NICOTINIC RECEPTORS.

Cecilia Bouzat
Instituto de Investigaciones Bioquímicas de Bahía Blanca, INIBIBB (CONICET-UNS), Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Bahía Blanca, Argentina.

The α7 nicotinic acetylcholine receptor is a pentameric ligand-gated ion channel. It is widely expressed in the central nervous system where it is involved in cognition, attention, and memory. It is also expressed in many non-neuronal cells and its activation has anti-inflammatory and neuroprotective roles. Enhancement of α7 activity is emerging as a therapeutic strategy for cognitive, neurodegenerative and inflammatory disorders. We have focused on understanding α7 function and its different mechanisms of modulation associated to physiological, pathological and therapeutic situations. By single-channel recordings we determined that positive allosteric modulators (PAMs) enhance α7 activation by increasing open-channel lifetime and inducing prolonged activation episodes, and we also identified novel PAMs. Although α7 has been considered the homomeric member of the family, heteromeric α7β2 receptors have been detected in human brain. We generated α7β2 receptors with different stoichiometries and determined how the β2 subunit modifies α7 kinetics and its allosteric modulation. This information is required to decipher the role of α7β2 receptors in native cells. In humans, there is a truncated α7 subunit (dupα7) that lacks part of the ACh-binding site and results from partial duplication of the α7 gene. We demonstrated that dupα7 acts as a negative modulator and can assemble with α7 into functional heteromeric receptors. Deciphering the molecular basis underlying α7 function has implications for the design of novel therapeutic compounds as well as for clarifying its pleiotropic actions.

6. IRON AND FERROPTOSIS IN AGING AND AGE-RELATED NEUROLOGICAL DISEASES.

Ashley Bush
The Melbourne Dementia Research Centre, The Florey Institute of Neuroscience and Mental Health and the University of Melbourne, Australia.

Recent research has implicated increased brain iron as a trait that can propel various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, Motor Neuron disease and the complications of stroke. Ageing itself causes iron to increase in the brain to a point where it is "too much of a good thing" and can set up conditions that lead to neurodegeneration. During childhood and reproductive life, iron recruitment is geared towards avoiding iron deficiency, but there is no natural mechanism for off-loading excess iron. After reproductive life the systems that harvest iron so efficiently do not turn off, and lead to accumulation in tissues that are not normally shed, like brain. In the C. elegans model of ageing, we find that such iron elevation limits lifespan. In Alzheimer's disease brain iron elevation is associated with the rate of cognitive loss, lipid peroxidation products and features of the regulated cell death mechanism, ferroptosis. Anti-ferroptosis agents have been effective in animal models of neurodegenerative disease, and a recent phase 2 clinical trial of the anti-ferroptotic chelator deferiprone in Parkinson's disease lowered nigral iron and improved clinical readouts. We are currently testing this drug in a phase 2 RCT in Alzheimer's disease. CuATSM, has recently reported benefits in phase 1 studies of Parkinson's disease and Motor Neuron Disease, and we have identified that it possesses potent anti-ferroptotic properties.

References:
Stockwell et al. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. Cell, 171, 273–285 (2017).
Ayton et al. Brain iron is associated with accelerated cognitive decline in people with Alzheimer pathology. Molecular Psychiatry, in press doi: 10.1038/s41380-019-0375-7.
Southon et al. CuII(atsm) inhibits ferroptosis: implications for treatment of neurodegenerative disease. British Journal of Pharmacology, in press.

SYMPOSIA

1. PURINERGIC SIGNALLING: FROM STRUCTURE-ACTIVITY TO APPLICATION IN PATHOLOGIES

ALLOSTERIC REGULATION OF THE P2X4 RECEPTOR CHANNEL GATING.

Stojilkovic, S.S.
Section on Cellular Signaling, Eunice Kennedy Shriver National Institute of Child Health and Human Development, NIH, Bethesda, MD, USA.

In general, allosteric modulators of ligand-gated receptor-channels induce conformational changes of the entire protein that alter potencies and efficacies for orthosteric ligands, expressed as the EC50 and maximum current amplitude, respectively. Recently, we studied the influence of allostery on channel gating using the rat P2X4 receptor expressed in HEK-293T cells and gated by ATP in the presence and absence of ivermectin (IVM), an established positive allosteric regulator of this channel. In the absence of IVM, this channel activates and deactivates rapidly, does not conduct NMDG+, a large organic cation, desensitizes completely with a moderate rate, and recovers only fractionally during washout. IVM treatment increases the efficacy of ATP to activate the channel, slows receptor desensitization during sustained ATP application and receptor deactivation after ATP washout, and makes channel permeable to NMDG+. Experiments with vestibular and transmembrane domain receptor mutants further established that IVM has distinct effects on the channel pore opening, the first accounting for increased peak current amplitude and the latter correlating with changes in the EC50 and kinetics of receptor deactivation. The corresponding kinetic (Markov state) models can reproduce many of the observed time series of evoked currents, as well as the transient changes in desensitization observed upon IVM application, the significant increase in ATP-induced current amplitudes at low IVM concentrations, and the modest increase in the unitary conductance. In summary, this study provides a detailed analysis of P2X4R kinetics and elucidates the mechanism regulating its channel gating.

CONTRIBUTIONS OF MOLECULAR DYNAMIC CALCULATIONS TO STRUCTURAL BIOLOGY UNDERSTANDING, THE CASE OF IVERMECTIN AND ALFAXOLONE AS P2X4R MODULATORS.

Huidobro-Toro J.P.; Latapiat V.; Alveal N.; Montenegro F.; Barrera N.
Laboratorio de Farmacología de Nucleótidos, Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile y Centro de Nanociencia y Nanotecnología, CEDENNA.

Multiple molecules modulate the electrophysiological activity of P2X receptors (P2XR). In particular, trace metals such as Cu(II) or Zn(II), modulate P2X4R concentrated in neurons by selective and specific cationic transporters; the putative site of action of these trace metals is apparently restricted to the extracellular receptor domain. Drugs such as the antiparasitic antibiotic ivermectin (IVN) or alfaxolone (A) a synthetic neuro-steroid, with hypnotic properties, also modulate the P2X4R but at intracellular sites, near the transmembrane P2X4R domain. In order to reveal and understand the site and mechanism of the modulator effect, as well as the allosteric agonism of A, we strategically used molecular dynamic simulations/ calculations to visualize the docking and mode of action of these modulators. A rP2X4R homology model was built; docking of either IVM or A revealed selective binding sites confined to the transmembrane domain as anticipated based on drug lipophilicity. In addition, molecular dynamic simulations in the APO and HOLO P2X4R states revealed allosteric-induced stability. Pore and lateral P2X4R fenestrations measurements of the different receptor states, in the absence and next in the presence of either IVM or A, showed that both IVM and A can elicit a larger pore opening that proved larger in the presence of ATP, as expected for allosteric modulators. Interestingly, in the case of A, consonant and consistent with an allosteric agonist, the hole analysis demonstrated an even larger opening. The present findings reveal the power and strategy of using simulation studies to understand molecular aspects of the binding and intricate molecular mechanism of allosteric agonist studies. Based on these findings, dynamic simulation calculations offer opportunities to design novel, P2X4R ligands not based on purines.

P2X2 RECEPTOR: NEW PHARMACOLOGICAL TARGET TO AD.

Fuentealba, J.
Depatamento de Fisiología, F. de Ciencias Biológicas, Centro de Investigaciones Avanzadas en Biomedicina (CIAB-UdeC), Universidad de Concepción, Chile.

Soluble oligomers of amyloid beta peptide (SOAβ) have been considered as central factors in Alzheimer's disease (AD). Aβ peptide is generated through the sequential cleavage of the amyloid precursor protein (APP), a process that requires the previous endocytosis of APP and that can be modulated by the multidomain adaptor protein Fe65. This protein is able to regulate the transcription of key genes directly related to AD pathogenesis, encoding proteins like APP and BACE 1. On the other hand, we have described that chronic SOAβ treatment induces an increase in the expression of the P2X2 purinergic receptor in PC12 cells and hippocampal neurons. Additionally, it has been described that the P2X2a isoform has an intracellular domain that can interact with Fe65, a segment which is absent on the P2X2b isoform. We found that SOAβ treated cells displayed an increase in evoked ATP currents (C: 100 ± 50%; SOAβ: 231 ± 70%; n=9). Additionally, immunocytochemistry (ICC) experiments demonstrated that these cells exhibited an increase in their P2X2R immunoreactivity (C: 100 ± 1 %; SOAβ: 149 ± 15%; n=5). Moreover, cells treated chronically with SOAβ showed a reduction in the Fe65 nuclear-cytoplasmic (N-C) ratio (C: 100 ± 6%; SOAβ: 80 ± 4%; n=5). A similar behavior was observed in PC12 cells transfected to express the P2X2a isoform, but not in those transfected with P2X2b (C: 100 ± 5%; P2X2a: 70 ± 6%; P2X2b: 95 ± 6%; n=3). Colocalization analyses demonstrated that SOAβ decreased the colocalization of Fe65 with APP (C: 100 ± 17%; SOAβ: 47 ± 12%; n=5); results that correlate with the increase observed in the colocalization of APP with clathrin (C: 100 ± 8%; SOAβ: 127 ± 8%; n=4) and Rab5 (C: 100 ± 6%; SOAβ: 132 ± 16%; n=5). In conclusion, these results suggest that chronic SOAβ treatment promotes the endocytosis of APP, potentiating its amyloidogenic processing. Additionally, the calcium dyshomeostasis/overload induced by P2X2R overexpression, alter the activation and localization of CAMKIIα, in the context of AD. Using molecular biology techniques, we observed that after chronic SO-Aβ treatments, mice hippocampal neurons

showed an increase on the levels of P2X2R compared to the control cells (C: 100.0 ± 6.4%; SOAβ: 130.1 ± 10.7%, n=5). This was correlated with increased Ca2+ signal evoked by ATP (C: 100.0 ± 12%, SOAβ: 194 ± 24%, n=4). Immunocytochemistry approaches on mice hippocampal neurons, showed that the overexpression of P2X2R induced changes on the immunorreactivity pattern of pCAMKIIα (in soma and neurites), which induced alterations on the cells morphology, and electrophysiological recordings assessed by Sholl Analysis and Patch Clamp, respectively. These results suggest that P2X2R overexpression can potentiate the toxicity of SO-Aβ, due to the chronic Ca2+ overload and inactivation of CAMKIIα, and thus, altering the mechanisms of neuronal plasticity, the basis of the pathophysiological mechanism of AD.

ROLE OF PURINERGIC SIGNALING AND IN GASTRIC CANCER.

Coddou C.1; Castro P.2; Cerda D.1; Reyna-Jeldes M.1; De la Fuente E.1.
1, Laboratorio de Señalización Purinérgica, Departamento de Ciencias Biomédicas, Facultad de Medicina, Universidad Católica del Norte, Coquimbo, Chile. 2, Departamento de Fisiología, Facultad de Ciencias Biológicas, Universidad de Concepción, Concepción, Chile.

Gastric cancer (GC) is the one of the most prevalent cancers worldwide. Here, we studied in detail purinergic signaling in several gastric adenocarcinoma-derived cell lines: AGS, MKN-45 and MKN-74, and compared them to a non-tumoral epithelial cell line: GES-1. In GC-derived cells, we detected the expression of several purinergic receptors, and found important differences as compared to GES-1 cells. Functional pharmacological studies with calcium imaging and proliferation assays revealed a strong contribution of P2YRs, especially P2Y2Rs to increases in cell proliferation and an antiproliferative effect induced by the activation of P2X4Rs. Also, we detected a tonic purinergic response that is probably a reflect of the paracrine and autocrine nucleotide signaling, because to sole application of purinergic antagonists changed the basal proliferation of GC-derived cells. In tumor-derived biopsies, we found an increase of P2Y2R and a decrease in P2X4R expression; however, we found high variability between the biopsies and their respective adjacent healthy gastric mucosa. Even so, we found a correlation between the expression levels of P2Y2R and P2X4R and survival rates of gastric cancer patients. Our latest experiments suggest that purinergic signaling also can contributes to epithelial to mesenchymal transition in CG-derived cells and that transactivation of P2Y2/HER2 can also contribute to these effects. Taken together, these results demonstrate the involvement of purinergic signaling in GC, and that the changes in expression and nucleotides release observed in GC could direct nucleotide signaling from anti-proliferative effects in healthy tissues to proliferative effects in cancer.

2. TOXICOLOGY AS A MULTIDISCIPLINARY SCIENCE

TOXICOLOGY AS A MULTIDISCIPLINARY SCIENCE.

Schulz B.
Toxicología, Departamento de Farmacia, Facultad de Farmacia, Universidad de Concepción.

The development of the Toxicology is strong related to the human history and its relationship with the environment, as well as geographic, social or politic environment. In the primitive phase of the toxicology as health topic, the principal focus of the knowledge was the description of the toxic events on humans, and its accidental or intentional character. Many of the early register of intoxications were based on the contact of plants, minerals or animals with the human bodies and it deleterious results. This description of events summarized the botanic, chemical, zoological and medical sciences. With the human enrichment on scientific information, the description of intoxications are more specific and included clinical symptoms, treatment of the intoxicated patients or forensic evidences in the body. For that are applied the physiopathology, forensic medicine, clinical medicine and pharmacology. The actual toxicology is related to many complex and heterogeneous knowledge, as well as analytical, molecular, computational, clinical, legal, epidemiological and pharmacological methods. In a more general aspect, toxicology is implicated in diverse applied areas, as clinical toxicology, forensic and legal toxicology, occupational toxicology, ecotoxicology and environmental toxicology. Therefore, toxicology is clearly a multidisciplinary science, it may be used in other areas, and toxicology needs information of other different disciplines as pharmacology.

STATE OF THE ART OF TOXICOLOGY IN CHILE.

Cavieres, F.
Toxicología, Facultad de Farmacia, Universidad de Valparaíso, Chile.

The first scientific meeting of Sociedad de Toxicología de Chile (SoTox) was held in november 2012. The event brought together toxicologists from academia, regulatory agencies and industry that represented the practice of toxicology in Chile. Only a few months earlier, SoTox had acquired its legal personality becoming Chile´s first scientific society in toxicology, so the meeting was an unprecedented opportunity to talk about the state of the art of basic and applied toxicological sciences in Chile. In general terms, it was acknowledged that toxicology played an important yet undefined role in the Chilean society, mostly due to the lack of professionals who had received formal academic training in toxicology. Seven years later, SoTox continues its efforts to position toxicology and toxicologists as an important discipline developed by professionals who can contribute to better decision making in the many different areas needed for the growth of the country. In this talk I will: i) introduce a modern perspective of what toxicology should be; ii) describe the state of development of the science in the country and iii) communicate SoTox´s goals and achievements.

EPIDEMIOLOGY OF INTOXICATIONS IN CHILE. 2018 ANNUAL REPORT OF THE TOXICOLOGICAL INFORMATION CENTER OF THE PONTIFICIA CATHOLIC UNIVERSITY OF CHILE (CITUC).

Silva, L.
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The epidemiology of poisonings in countries is relevant when addressing public health guidelines, evaluating exposure profiles and defining treatment and prevention strategies. This presentation describes the characterization of exposures to potentially dangerous substances based on the cross-sectional descriptive study of the universe of calls that entered the CITUC toxicological emergency center during 2018. The variables analyzed were: sex, age, circumstance of the exposure, agent (s), interlocutors of the call, location of the interlocutor and the incident, routes of exposure, symptomatology and severity. This work collects information regarding calls for toxicological emergencies that entered CITUC, from the 15 regions, 54 provinces and 346 communes of Chile. During the telephone call, the emergency center professionals collect all available information provided by the caller, required for the evaluation of the case considering data of the agent, the circumstances of the exposure and the patient. After the background evaluation, the technical recommendations for exposure management are communicated. The data is collected in the manual registration form and subsequently all the data is entered into the electronic Registration System called "Call Registry System CITUC SRL". The Central of toxicological emergencies provides free telephone assistance with qualified professionals 365 days a year in continuous hours (24/7), answering questions from health professionals, authorities, emergency personnel and the general public. The commitment and dedication of the professionals, together with the excellence in the service, guarantee the 27 years of existence of the center and the 35,000 calls on average that CITUC receives annually.

CLINICAL MANAGEMENT OF POISONINGS: SPECIALISTS NEEDED.

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In Chile, the number of acutely poisoned patients, admitted into emergency medicine departments, is increasing every year. Under this circumstance, medical staff need to be prepared, in terms of proving poisoned patients, with adequate clinical treatment. According to the National Poison Control Center (CITUC), 57% of phone calls received at the center are from healthcare settings. This may be indicative of existing limitations related to the clinical management of poisoned patients. Thus, availability of basic up-to-date knowledge about clinical management of poisoning events would optimize treatment regimes, economic burden, and also improve patients' prognosis. A classic example of this is the use of gastric decontamination techniques, such as gastric lavage and activated charcoal, when patients expose to chemicals through the oral route. However, there are some limitations when employing these maneuvers. Specifically, the frame time elapsed between the exposure and the use of the technique (up to 60 minutes). After that time, there is a significant decrease of effectiveness, and the procedure

itself turns, not only very traumatic to the patient, but also in unnecessary expenses to the healthcare setting. This inappropriate practice has become common in several health institutions, which are characterized by a lack of specialized medical staff with knowledge in clinical toxicology.

3. TRANSLATIONAL OPTIONS FOR THE TREATMENT OF DRUG-ABUSE DISORDERS: OPPORTUNITIES, SUCCESSES AND PITFALLS

INTRODUCTORY REMARKS.
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Synthesized molecules and drugs can be used for rapidly achieving a high pleasant and/or euphoric mood, bypassing the homeostatic pathways for pleasure and reward. That can be escalated by repeating and increasing the drug experience, abusing of the shortcutting pathway to pleasure, arriving to dependence and addiction to the substances providing that shortcut, changing the physiological substrate for perpetuating an addicting behaviour, impacting on the social and familiar environment for the compulsion of "experiencing a new trip". Much has been investigated about the physiological and neuronal framework sustaining that condition in mammals, including humans, arriving to the pivotal neurocircuitry of pleasure, identifying a role for dopamine, glutamate and opioid neuropeptides. The obtained knowledge is enormous, but that has not led to a consensus for treating a medical issue, which is not only menacing the individual, but is destroying the society. We have hereby sampled a group of international leaders and experts, who have devoted a research life to investigate the issue, both at basic and clinical levels, to discuss why preclinical results have not translated into the clinic. Thus, Gaetano Di Chiara (Cagliari), who first proposed a role for accumbens dopamine release as a common substrate for addictive substances will discuss about translational approaches for treating cocaine addiction. Rainer Spanagel (Mannheim), a leader on neuropeptides and addiction will discuss on the role of corticotropin-releasing pathways for sustaining drug abuse and addiction. Yedy Israel (Santiago), an international referent on alcohol and alcoholism will discuss on a neuroinflammatory-oxidative stress cycle sustaining chronic alcohol intake.

TRANSLATIONAL APPROACHES TO THE TREATMENT OF COCAINE ADDICTION.

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Cocaine addiction treatment is probably the most challenging and paradigmatic example of the difficulties in translating neurobiological knowledge into addiction therapy. Thus, to date, in spite of the advances in the knowledge of the neural basis of cocaine addiction, none, among the translationally-based treatments proposed, has been approved by national or international agencies. Clearly, the difficulties with cocaine addiction might be a case of a general difficulty in translating experimental results obtained in animals into human therapy. Although drug addiction is recognized as a brain disease, it is an exceedingly complex one and it is unclear to which extent, as in the case of schizophrenia and dementia, animal models are able to model the human condition. Therefore, critical analysis of the defaillances with cocaine can provide important clues as to the models to utilize. As far as the main lines of research, simple approaches targeting DA transmission, the indirect site of cocaine action, with DA receptor agonists and antagonists or with low abuse liability DAT blockers have been discouraging, although the development of allosteric DAT ligands is currently a major line of research at NIDA. Acknowledgment of the critical role of neuroplastic changes at the level of the glutamatergic/dopaminergic cortico-ventral striatal circuit is the basis for pharmacological and physical treatments (DBS and TMS), aimed at reversing the neural changes induced by long lasting drug exposure. Preliminary, small scale observations in humans indicate that this might be the right way to go.

CORTICOTROPIN-RELEASING HORMONE RECEPTOR 1 (CRHR1) AND ADDICTION: WHY THE PRECLINICAL RESULTS DID NOT TRANSLATE INTO THE CLINIC?.

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ALCOHOL-INDUCED NEUROINFLAMMATION-OXIDATIVE STRESS CYCLE: INFLAMMATORY AND ANTIOXIDANT DRUGS INHIBIT CHRONIC ALCOHOL INTAKE AND BLUNT RELAPSE BINGE-DRINKING.

Israel Y. 1, Quintanilla ME.1, Ezquer F.3

, Morales P. 1,2 ,Ezquer M. 3, Herrera-Marschitz,M.1.
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Brain of UChB rats chronically consuming over 10 g ethanol/kg/day show (i) a 200% increase in hippocampal oxidative stress, determined as the ratio of oxidized/reduced glutathione (GSSG/GSH), and (ii) marked neuroinflammation, shown as 60% increases in astrocyte glial-fibrillary acidic protein (GFAP) and increases in microglial density (Iba-1). Noteworthy, these changes remain long after ethanol intake is discontinued; in line with a proposed self-perpetuation (vicious cycle) of oxidative stress and neuroinflammation. Administration of a low dose of the antioxidant N-acetyl cysteine (40 mg/kg) reduces brain oxidative stress and neuroinflammation and inhibits chronic alcohol intake by 50-60%. The co-administration of N-acetyl cysteine with low doses of aspirin (ASA 15 mg/kg) inhibit alcohol intake by 75%, showing a significant synergism of both drugs. Following chronic ethanol intake, co-administration of N-acetyl cysteine plus the anti-inflammatory drug during a 2-week alcohol deprivation period block neuroinflammation and oxidative stress and inhibit by 85% the relapse binge-like drinking ("ADE") prompted by the subsequent ethanol re-access. As will be shown, studies tie neuroinflammation-oxidative-stress and hyper-glutamatergic conditions as the likely mechanisms that perpetuate chronic alcohol intake and promote intoxicating relapse drinking, and also indicate the pharmacological agents that block this condition. Studies suggest that anti-oxidant and anti-inflammatory agents may add significantly to interventions aimed at reducing alcohol-use disorders.

4. STRATEGIES FOR THE DESIGN AND DEVELOPMENT OF NEW DRUGS

DRUG DESIGN STRATEGIES BASED ON THE MOLECULE.

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Resistance to antibacterial agents is a growing problem of global public health, which affects the treatment of infectious diseases, reducing the effectiveness of available antibacterials, causing an increase in mortality and morbidity of patients. If innovative initiatives that seek to solve this problem are not generated, it is projected that, worldwide, in the year 2050 there will be around 10 million deaths caused by resistant microorganisms, with costs estimated at 100 trillion dollars. Unfortunately, the pharmaceutical industry has been leaving research in this area which is reflected in the approval of only ten drugs by the FDA, during the last ten years, none of them with new mechanisms of action. Due to this is why it is necessary to promote and encourage the development of a greater quantity of antibacterial compounds, different from those already known. For this reason, the discovery and development of new molecules is of vital importance. Thus, different approaches have been used for the discovery of active compounds, such as the High-throughput screening, the extraction of compounds from natural products or the design based on a biological target. But, what can be done when an isolated molecule (or series) is available and its pharmacological target is unknown? How to know which area of the molecule can be modified and what type of modification can be made to obtain compounds that are more active? How to know the relationship structure activity of this family of molecules? In this work, we show the experience of development of a new family of antibacterial drugs using different strategies of traditional medicinal chemistry. References W. H. Organization, Antimicrobial resistance: global report on surveillance. World Health Organization, 2014. J. Campanini-Salinas, J. Andrades-Lagos, J. Mella-Raipan, and D. Vasquez-Velasquez, "Novel classes of antibacterial drugs in clinical development, a hope in post antibiotic era.," Curr. Top. Med. Chem., 2018. J. Campanini-Salinas et al., "A New Kind of Quinonic-Antibiotic Useful Against Multidrug-Resistant S. aureus and E. faecium Infections," Molecules, vol. 23, no. 7, 2018.

INTEGRATION OF STRUCTURAL BIOINFORMATICS AND CHEMICAL BIOLOGY FOR THE DISCOVERY OF NOVEL DRUGS.

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The discovery and design of drugs is increasingly incorporating structural bioinformatics techniques to model and analyze proteins of biological or therapeutic interest, perform large-scale virtual screening programs to identify lead compounds and evaluate molecular interactions through molecular dynamics simulations. These techniques are fast, cost effective and complementary to the existing experimental techniques of chemical biology. In this presentation, we will discuss some examples of strategies that combine different structural bioinformatics approaches with chemical biology tools to successfully discovery of novel drugs, focusing on the analysis of the inherent strengths and limitations on the use of structural bioinformatics tools, as well as complementary biological assays.

DESIGN STRATEGIES FOR NEW CLASSES OF ANTIBACTERIAL DRUGS, A HOPE IN A POST-ANTIBIOTIC ERA.

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Bacterial resistance is a growing problem worldwide and is estimated that deaths by infectious diseases associated with resistant pathogens will generate 10 million deaths per year in 2050. This problem becomes more serious due to the low level of research and development of new drugs, which has fallen drastically in the last 40 years. For example, in the last decade of a total of 293 new drugs approved by FDA, only 9 corresponded to antimicrobial drugs and none constituted a new structural class. The majority of the molecules in the clinical phase II or III, coming from modifications of drugs in clinical use, this strategy make easier the bacterial susceptibility to generate resistance through the mechanisms expressed for their drug predecessors. Under this scenario, is urgent to generate the most novel strategies for the development of antibacterial compounds with new targets or mechanism of action, without structural relationship with the antibiotic drugs predecessors. Under this look, the present work addresses the development of the latest antibacterial drugs in clinical phases II and III, analyzing the design strategies by which these new molecules were obtained and the structure-activity relationship of these new families of antibiotics, in order to define the state of the vanguard antibacterial drugs in the post-antibiotic era.

PRECLINICAL DEVELOPMENT OF A NEW CLASS OF ANTIBACTERIALS, A NATIONAL EXPERIENCE.

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The rapid emergence of resistant bacteria is occurring worldwide, endangering the efficacy of antibiotics, reducing the therapeutics arsenal for treatment of infectious diseases. According this, it is a urgent need the development of new antibacterial drugs. In this study, we develop a new class of compounds obtained with a simple synthesis in two single-step. These compounds were screened for in vitro antibacterial activity against ATCC strains and clinical isolates, using the broth microdilution method. In addition, a series of trials were conducted to gather information about the effectiveness and safety of derivatives, such as how; toxicity in mammalian cells and galleria mellonella, assays of potential for induction of mutations, among others. The compounds exhibited MICs of 1-32 μg/ml against Gram-positive ATCC strains. The MIC50 for compound 7 against the MRSA isolates tested were 2 mg/L, compound 16 exhibit 2 mg/L. For the VREF isolates the compound 7 showed MIC50 and MIC90 values of 2 and 4 mg/L, and the compounds 16 obtain values of 4 and 4 mg/L. The compounds were bactericidal in all isolates tested. Both compounds were bactericidal in all clinical isolates tested. Neither compound affected cell viability in any of the mammalian cell lines and Galleria mellonella larvaes, at any of the concentrations tested. These in vitro data indicate that compounds 7 and 16 can advance in assay on murine models of infection and determination of pharmacokinetics parameters.

5. ALZHEIMER'S DISEASE: NEW TARGETS AND DRUGS.

INTRODUCTION.

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Alzheimer's disease (AD) is becoming a devastating health, social, and economical problem. Burden to society will increase as population ages. The search of disease-modifying drugs has focused on over 30 distinct targets, most of them linked to amyloid beta (Aβ) aggregation or hyperphosphorylated tau. Anti-oxidant, anti-inflammatories, neurotransmitter receptors, or growth factors have been explored as targets to develop a medicine to delay disease progression. Ligands for those targets have been explored in cell and murine models of AD. Although many of them have shown efficacy in this preclinical set-up, they have failed in dozens of clinical trials performed during the last 20 years in AD patients. It is interesting that the Alzheimer's Foundation for Drug Discovery (AFDD) is not supporting any more clinical trials with compounds targeted to Aβ or tau. So, new targets and ideas are urgently needed. In this symposium on new targets and drugs for AD, four scientists from the Institute "Teófilo Hernando for Drug Discovery", at the Universidad Autónoma de Madrid, Spain, will present their work on new approaches to the search of new targets beyond conventional (Manuela García López), multitarget compounds (Rafael León Martínez), and Phosphatase PP2 (Raquel López Arribas). A last communication focus on altered neurotransmission processes in AD (Luis Gandía Juan). It is expected that only with these and other new strategies, we can find out the way to a medicine capable of slowing down the natural course of the disease; and what it is even more challenging, if administered at presymptomatic AD stages, in patients at risk diagnosed with biomarkers, this medicine be capable of delaying disease

NON CONVENTIONAL TARGETS FOR THE TREATMENT OF ALZHEIMER´S DISEASE.

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Alzheimer´s disease (AD) is the most common form of dementia with still no effective treatment. From a histopathological point of view, AD is characterized by extracellular aggregates of betaamyloid and, intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein. During the last twenty years great effort has been made to develop therapeutic strategies, mostly based on beta-amyloid pathology, but without success. On the other hand, AD shares with other neurodegenerative diseases pathological mechanisms like oxidative stress, subchronic inflammation, mitochondrial dysfunction and proteinopathy. Given this scenario, our group seeks to identify new therapeutic targets focused on the regulation of oxidative stress and neuroinflammation, processes that precede the accumulation of aberrant proteins and cognitive impairment. We have therefore centered out attention on two targets: (1) the NADPH oxidases enzymes (NOXs), which are the enzymes responsible for the production of reactive oxygen species such as superoxide and hydrogen peroxide, and more specifically, in its NOX4 isoform and, (2) in the transcription factor NRF2 (Nuclear factor (erythroid-derived 2) -like 2), master regulator of the antioxidant response, which also regulates the expression of genes that participate in the anti-inflammatory response and autophagic processes. To validate NOX4 as a possible target, we have used transgenic mice that do not express this enzyme and a model of taupathy by injecting i.c.v. adeno viruses containing the human tau protein mutated in P2301L, under the promoter synapsin. In this model we have been able to determine that animals that do not express NOX4 have less oxidative stress and less neuroinflammation which results in an improvement in the cognitive tests. For our second target, we are looking for compounds that inhibit the interaction Keap-1 (NRF2 repressor protein) and NRF2. To do this, we have performed an in-silico screening of large libraries using docking and molecular dynamics, as well as the synthesis of new compounds. In the latter case, we want to obtain multitarget compounds with complementary activities to the induction of

NRF2, with the aim of interfering on different nodes of Alzheimer's pathophysiology. In this project we are following a sequential screening protocol based on studying the Nrf2 induction, antioxidant, anti-neuroinflammatory and neuroprotective properties of the compounds. As a last step, those compounds with a more favorable toxicological, pharmacokinetic and pharmacodynamic profile will be evaluated in in vivo models of AD.

ALTERATIONS IN NEUROTRANSMISSION RELATED TO THE PROGRESSION OF ALZHEIMER'S DISEASE.

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Alzheimer´s disease (AD) is the most common form of dementia, being aging the main risk factor for the development this disease. The alteration of several neurotransmitter systems has been reported in AD, which could be correlated with changes in the synthesis, storage or release of these neurotransmitters. In this study, we tested how aging affects ionic currents, cell excitability and last steps of exocytosis under physiological and pathological conditions. For this purpose, we used a triple transgenic model of AD (3xTg-AD) that contains mutations in the gene encoding the amyloid precursor protein (APPSwe), presenilin-1 (PS1M146V) and tauO301L, which mimics the development of the disease on Alzheimer's patients, and B6129SF mice (wild type). By using amperometric techniques, we have found significant changes in the exocytosis of catecholamines occurring in mice of 6 and more than 12 months of age, where the pathology is already established, when compared with prepathological mice (2 months), in particular, an increase of the number of amperometric spikes, although the quantal catecholamine content on individual spikes is lower. Kinetic analysis of secretory spikes shows that as the disease progresses amperometric spikes are faster in triggering and shorter in duration. Patch-clamp technique was also used to measure the different ionic currents involved in the physiological release of catecholamines. We observed a decrease in sodium currents and an increase in potassium currents in 3xTg-AD compared with controls. Nicotinic currents exhibited a similar pattern throughout the age in both control and transgenic mice. Finally, we found an increase in calcium currents in 3xTg-AD mouse with age that was not observed in wild type mice. These findings suggest that throughout the development of 3xTg-AD mice and as Alzheimer's disease is established there is a change in chromaffin cell excitability, which causes neurotransmission to accelerate. These alterations could have an impact on the response that the organism offers in a stressful situation.

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NEW COMPOUNDS DIRECTED TO PP2A, A PROMISING THERAPEUTIC TARGET FOR ALZHEIMER'S DISEASE.

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Introduction: Alzheimer's disease (AD) is the most common cause of dementia. Nowadays, there is no cure for AD or a way to stop or slow its progression. Main histopathological hallmarks of AD are senile plaques, and neurofibrillary tangles, generated by aggregation of the microtubule associated protein tau. Over the past two decades, most scientific efforts in the development of new drugs to treat AD have focused on inhibiting the degradation of acetylcholine and avoiding amyloidogenesis. However, less interest has aroused the therapeutic approach consisting in preventing neurofibrillary death by inhibiting the abnormal hyperphosphorylation of tau. In this sense, pharmacological strategies have been almost completely oriented to inhibit the activity of tau kinase enzymes, with discouraging results. Our research group proposes to address the aberrant phosphorylation of tau by restoring the activity of its main phosphatase enzyme, protein phosphatase 2A (PP2A), which is decreased in the brains of patients with AD, mainly due to the increase in the expression of the endogenous inhibitors I1PP2A and I2PP2A/SET . Hypothesis: The study of the structure-activity relationship of okadaic acid (OA), a toxin with selective inhibitory activity of PP2A, has allowed us to design and synthesize new analogue molecules of

OA that lack such inhibitory capacity. In this sense, our starting hypothesis states that these compounds, due to their binding to the catalytic subunit of PP2A, would be able to compete with the endogenous inhibitors of PP2A, and thus, to restore the compromised phosphatase activity in AD. Material and results: Our molecules are able to reduce OA-induced neurotoxicity and some of them also present a good profile in a model of oxidative stress in SH-SY5Y cells and in a model of excitotoxicity in cortical neurons. The new compounds maintained serine/threonine phosphatase activity, depressed by the action of two PP2A inhibitors: OA and cytostatin. Molecular docking studies indicated that the compounds studied are capable of binding PP2A in a similar manner to OA, but does not interact with the catalytic site, confirming our initial hypothesis. Conclusions: Our compounds have a potential indication for the treatment of neurodegenerative diseases based on the maintenance of PP2A activity, which avoids tau hyperphosphorylation.

6. MEDICINAL CHEMISTRY: RATIONAL DESIGN AND STRUCTURE ACTIVITY RELATIONSHIP, A SYNTHETIC APPROACH OF NEW BIOLOGICALLY ACTIVE SUBSTANCES.

SYNTHESIS OF NEW INDOL DERIVATIVES AND THEIR ACTIVITY ON CHOLINERGIC AND SEROTONERGIC SYSTEMS AND IN BETA-AMYLOID DEPOSITION. A MULTIFUNCTIONAL APPROACH TO ALZHEIMER'S DISEASE.

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Alzheimer's disease is the most diffuse form of senile dementia, and is among the most devastating brain disorder an individual can face. It involves progressive and irreversible decline in cognitive functions including memory, judgment, decision-making, orientation to physical surroundings and language. Despite substantial efforts in drug development and an increased understanding of the underlying pathology of Alzheimer's disease, no effective treatment has yet been achieved. The main goal of this research is to contribute to the knowledge of the medicinal chemistry in the neurochemistry field, through the design of novel ligands functioning as multitarget agents in Alzheimer' disease (AD). In this proposal, we describe the synthesis and in vitro biological evaluation of novel indole derivative as single chemical entities to simultaneously modulate multiple targets which comprises i) binding-affinity and potential agonist properties of serotonin 5-HT4R ii) acetylcholinesterase inhibition, iii) serotonin transport re-uptake inhibition and iv) inhibition of β-amyloid deposition.

DESIGN OF NEW BENZIMIDAZOLES WITH BETA-3 ADRENERGIC AFFINITY.

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The human receptor β3-adrenergic has been the target of recent studies due to its potential to modulate various physiological aspects of the organism involved in numerous pathologies such as diabetes, hypertension, overactive bladder, heart problems, depression, and cancer, among others. In this context, our efforts are focused on the design, synthesis and biological evaluation of new heterocyclic compounds capable of binding to the human receptor β3-adrenergic. Since the beta-3 receptor is not crystallized, we have performed extensive studies based on ligands (3D-QSAR, CoMFA, and CoMSIA), which have allowed us to generate a pharmacophoric model that we use as a basis for the rational design of our compounds. The routes of synthesis of the heterocycles proposed by our group follow a similar route to that used to obtain Mirabegrón, the only drug currently available that acts on the beta-3 receptor indicated in the treatment of overactive bladder.

NEONICOTINOIDS, SEARCHING FOR NICOTINIC RECEPTOR LIGANDS OF ALPHA4BETA2 NACHR SUBTYPE AND ITS APPLICATION AS NEW ANTI-ADDICTIVE SUBSTANCES.

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Addiction is a chronic and compulsive drug seeking, producing detrimental consequences, and long-lasting changes in the brain. It is

considered a brain disorder and a mental illness. Addiction is the most severe form of substance use disorders, caused by repeated misuse of a substance. Neuronal Nicotinic Acetylcholine Receptors (nAChR) are involved in nicotine addiction and emerging evidence suggests that nAChR could be acts as pharmacological target to be considered in alcohol abuse. Two of the most common addictive substances used and accepted by the society. Nicotinic ligands have been designed, synthesized and tested on nicotinic receptor for decades, but the focus of the design has been full and partials agonists with good therapeutics results on nicotine addiction (i.e. cytisine and varenicline). However, there are little evidences indicating that nicotinic antagonist could expert anti-addictive effects over nicotine addiction and alcoholism. In our lab, we had designed and synthesized simple nicotinic analogues with agonist or antagonist properties on alpha4beta2 nAChR subtype and using zebrafish as a behavioural model we have identified a new nicotinic antagonist, named UFR2709 that revert the effect of nicotine using a homologous CPP for zebrafish. Additionally, we tested UFR2709 on Wistar-derived University of Chile alcohol-preferring UChB rats a well-known model to evaluate ethanol consumption. Our results show that UFR2709 are able to decrease the ethanol intake using a two-bottle choice paradigm assay. UFR2709, an alpha4beta2 nAChR antagonist shows an anti-addictive effect on nicotine addiction and ethanol consumption and open a new way for drug design and the treatment of nicotine and ethanol addictions.

7. ANTIMICROBIAL ACTIVITY OF HERBAL EXTRACTS AGAINST CLINICALLY RELEVANT PATHOGENS

NATURAL EXTRACTS AND THEIR ROLE IN THE SEARCH FOR NEW THERAPEUTIC ALTERNATIVES TO TREAT INFECTIONS.

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In May 2015 the World Health Assembly adopted a global action plan against antibiotic resistance, since deaths related to multidrug resistant bacteria have increased in alarming speed in the last decades. One of the goals of this plan is to support research and development of new antimicrobial drugs, since classic or conventional antibiotic drugs have been proposed to become obsolete in a few decades from now. This scenario is not exclusive for bacterial infections, since the lack of new clinically effective drugs is also a problem for fungal and parasite infections. In this context, natural products have emerged as a valid alternative for the discovery of new antimicrobial agents with new mechanisms of action and in some cases even in absence of current resistance mechanisms. Plants are affected by several microorganisms, so they count with high content of secondary metabolites with antibacterial, antifungal and antiparasitic effects such as flavonoids, tanins, terpenoids and alkaloids. However, herbal extracts can vary among the same species due to different extraction methods, different geographical location and even season collection. So, it is important to count with adequate characterization methods in order to achieve reproducible results and standardized extracts respect to their chemical composition and the proportion of active principles that can be related to their antimicrobial activity.

REVEALING ANTIMICROBIAL ACTIVITY OF NATURAL PRODUCTS USING CHEMICAL SUBTRACTION AS NEW STRATEGY TO PREPARE KNOCK-OUT AND KNOCK-IN EXTRACTS.

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Chilean plants have biased and incomplete chemical-pharmacological studies. The main reason for that has been the low availability of enough quantities to make biological tests and structural elucidation. Moreover, the isolation of specific constituents often omits the residual complexity existing in plants, in which it is not uncommon to find highly active compounds. In this work is presented the application of a new strategy to investigate antimicrobial activity of medicinal and food plants. This tool unifies different pharmacological/phytochemical approaches using the liquid-liquid methodology named Centrifugal Partition Chromatography (CPC) to obtain DESIGNER extracts. These extracts could be “knock-out” (selective removal of one or a group of compounds) or “knock-

in" (selective addition of one or a group of compounds). In the first example, we prepare "knock-out" from propolis and Buddleja globosa (Matico) and assess their antimicrobial activity. Propolis without caffeic acid phenyl ester (CAPE) shown similar antimicrobial activity compared to raw extract, suggesting that other compounds present in its residual complexity are responsible for such activity. On the other hand, Matico "knock-out" (selective removal of verbascoside), displayed minimal antimicrobial activity. In this last example, the re-incorporation of verbascoside recovered the biological activity. Finally, we perform a double knock-out in Peumus boldus extract, removing cytotoxic compounds (e.g. ascaridole) and isoquinoline alkaloids (e.g. boldine). This Boldo DESIGNER extract reduce significantly cell injury in H. pylori-infected AGS cells without the cell toxicity observed in the raw extracts. To confirm the protective properties of this extract in vivo, we used a continuous liquid-liquid separation by True Moving Bed system (TMB-500). Hence, a dose of 100 mg/kg/day of Boldo DESIGNER extract was able to prevent H. pylori SS1 infection in Mongolian gerbils.

PHYTOPHARMACEUTICALS AND ANTIMICROBIAL RESISTANCE IN VETERINARY MEDICINE.

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There is an increase in fungal infections owing to the appearance of resistant fungi to different drugs. Candida albicans is part of the resident microbiota of the oral cavity but is also the most frequent fungal pathogen, whose biofilms formation represents one of the main resistance mechanisms. In the oral cavity, Candida albicans biofilms are extremely resistant to antifungals and together with the absence of new, effective and safe antifungals, the search for pharmacological alternatives is warranted. It has been described that essential oils from Lavandula dentata, and endemic plant in Chile, possess antimicrobial and antifungal activity against several microorganisms including Candida albicans. We described the antifungal and antibiofilm effect of Lavandula dentata essential oil on the inhibition of Candida albicans Fluconazole-resistant strain (ATCC 10231), to adhere to abiotic surfaces and to form biofilms. After the chemical characterized of the essential oil by Gas Chromatography and the determination of minimal inhibitory concentration (MIC), we evaluated the effect of this essential oil on the adhesion ability through crystal violet assay and the antibiofilm effect through the viability of biofilm formation and scratch assay. The MIC was able to inhibit adhesion and biofilm formation in an abiotic surface for the resistant strains assayed (ATCC 10231). In conclusion, this study demonstrates that this essential oil from Lavandula dentata could be a promising strategy against biofilms from resistant Candida albicans strains. Since phytodrugs present many active compounds, who makes then difficult to generate resistance, they can be used in conjunction with conventional antifungal, sensitizing the pathogens and decreasing its adhesion and later formation of biofilms.

PARASITES AND PLANTS: ELUCIDATING THE ANTIPARASITIC ACTIVITY OF CICHORIUM INTYBUS (CHICORY).

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Parasitic helminths and protozoa affect billions of people worldwide and are amongst the most prevalent infections in livestock. Due to increasing parasite drug resistance towards the limited therapeutic arsenal available, novel antiparasitics are urgently needed. Plants with reported antiparasitic activity have been traditionally used and may provide new lead compounds. One antiparasitic plant increasingly investigated is chicory (Cichorium intybus; Asteraceae), a perennial herb distributed worldwide and commonly cultivated as crop for human and livestock consumption. Chicory has attracted research interest for its effects against parasitic nematodes in livestock, which have been linked with its content of sesquiterpene lactones (SLs). Previous in vivo studies have confirmed that chicory-fed animals have a reduced parasite burden, but detailed identification of responsible compounds has not been, until recently, thoroughly explored. By integrating parasitological studies and metabolomic analyses, we have investigated the anthelmintic activity and phytochemical profile of SL-extracts from chicory material sampled in different geographical regions. The in vitro activity of chicory SL-extracts was first evaluated using the free-living nematode Caenorhabditis elegans model and further confirmed in the parasitic pig nematode Ascaris suum, which is closely related with the human parasite A. lumbricoides. Marked differences in anthelmintic potency

were observed between SL-extracts from different chicory material. Bioactivity-based molecular networking analyses suggest that some but not all SLs are linked with the anthelmintic activity of chicory. In addition, we have explored the antiprotozoal activity of chicory against Trypanosoma cruzi, the etiological agent of Chagas disease. Chicory SL-extracts induced potent concentration-dependent trypanocidal activity against T. cruzi trypomastigotes at concentrations that are not toxic to mammalian cells. Isolation and testing of individual chicory SLs are undergoing to evaluate their antiparasitic mechanisms.

8. BIOTECHONOLOGY ASPECTS OF ASPARAGINASE CLINICAL AND INDUSTRIAL DEVELOPMENT

PRODUCTION OF EXTRACELLULAR L-ASPARAGINASE: FROM BIOPROSPECTING TO THE ENGINEERING OF AN ANTILEUKEMIC BIOPHARMACEUTICAL.

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In 2013 the production of L-Asparaginase, a biopharmaceutical widely used in the treatment of acute lymphoblastic leukemia (ALL), was suspended by the foreign manufacturer who supplied the drug (Elspar®) to Brazil since the 1980s. The interruption of this supply led to life threatening delays in treatment which forced the Brazilian Ministry of Health to find emergency alternatives, including importation of a more expensive alternative (Aginasa®). Later, in 2017 and following an international public tender, MS started importing the medicine Leuginase®, from China. Such frequent changes in the supply of L-Asparaginase has provoked intense and controversial debate in Brazil regarding the quality of the imported medicine. This motivated our group to develop a technology platform for the production of L-asparaginase with more advantageous characteristics than the imported formulations. Brazil is considered a weak player on the World stage in biopharmaceutical discovery, development and production of biopharmaceuticals, and the present project proposes a union between several scientific and technological competences for the development of industrially viable L-Asparaginase production process. This new proposal is a continuation of the FAPESP Thematic Project (2013 / 08617-7) that has provided promising results, since it enabled the development of new recombinant strains of bacteria and yeast with the capacity to produce L-asparaginases with longer half-life, greater stability, lower toxicity, and lower side effects in comparison to the biopharmaceuticals currently in clinical use not just in Brazil, but worldwide. As a continuation of the previously initiated studies, this thematic project proposes the development of processes for the production, under GLP and GMP of 4 antileukemic biopharmaceuticals with different characteristics and with important potential to be produced nationally and even for export, including: 1) Escherichia coli BL21 (DE3) – a recombinant wild-type E. coli ASNase, overexpressed in epichomal vector pet28a with a resistance marker for kanamycin; 2) Escherichia coli BL21 (DE3) – a recombinant wild-type ASNase from Erwinia chrysanthemi ASNase, overexpressed in epimasomal vector pet28a with a resistance marker for kanamycin; 3) Escherichia coli BL21 (DE3) – a recombinant E. coli ASNase resistant to human serum proteases - overexpressed in epichomal-vector pet28a with a resistance marker for kanamycin; 4) Recombinant Pichia pastoris – a recombinant wild-type E. chrysanthemi crisantaspase with humanized glycosylation (expressed in pJAG-s1 in the Superman5-Glycoswitch yeast from Biogrammatics™). To improve aspects of stability, bioavailability, toxicity and hyperallergenicity, which are problems observed with current formulations, nanotechnological approaches such as pegylation and encapsulation in polymer vesicles will be used. The project aims to develop a production process from optimization of microbial cultures to purification, pegylation and final formulation (lyophilized product), in sufficient quantity to carry out subsequent preclinical studies. The entire study will be accompanied by technical and economic evaluations (Quality by Design).

DEVELOPMENT OF BIOTECHNOLOGICAL PROCESS FOR THE PRODUCTION OF THE ANTILEUKEMIC BIOPHARMACEUTICAL L-ASPARAGINASE (ASNASE) USING GENETICALLY MODIFIED MICROORGANISMS.

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There is a strong global tendency to find alternative ways to produce pharmaceutical active principles from biotechnology process. In this scenario, Latin American countries show a small expression in both research and production. Worsening the situation, international suppliers of biopharmaceuticals to this region are losing interest in the market and discontinuing production, especially those related to onco-hematologic treatment. In this context, the union of different scientific and technological skills have joined to achieve a viable industrial process to biotechnologically produce L-Asparaginase, a biopharmaceutical broadly used in the treatment of leukemia. Two major research fronts are being studied with the objective of finding a promising antileukemic biopharmaceutical: the optimization of endogenous and heterologous production processes of the enzyme, with bioprospecting groups of fungi of the most varied biomes; and the rational engineering of proteins that will utilize as scaffold the S. cerevisiae and E. coli L-Aparaginases for comparative studies with the bacterial isoforms currently employed in therapeutics. To improve aspects of stability, bioavailability, toxicity and allergenicity, problems observed with bacterial formulations, several nanotechnological approaches are being used, such as pegylation and encapsulation in polymeric vesicles, and the project aims to generate a biopharmaceutical to be produced industrially. Fungi from different biomes, such as cerrado, caatinga, marine environment, and Antarctica, have been isolated and several of them have been evaluated for the production of the enzyme in shaker and in 3- or 7-Liter bioreactors, and by solid state cultivation. Biochemical and kinetic characteristics are being determined for all isolated L-asparaginases. The studies aim at obtaining recombinant E. coli and Pichia pastoris to produce of L-asparaginase with potentially improved characteristics (longer half-life, higher stability, lower toxicity and lower side effects) in comparison those that are in clinical use in the World. A recombinant E. coli with the capacity to produce L-asparaginase resistant to two plasma proteases was obtained and the toxicity studies shows important potential for starting preclinical studies. A recombinant P. pastoris strain with the ability to produce L-asparaginase with humanized glycosylation was also obtained, with great potential to reduce to immunogenic reactions and, therefore, safer for patients. Pegylation and nanoencapsulation studies of the novel L-asparaginases are being conducted and the results have shown that site-directed pegylation has the potential to generate a biopharmaceutical with better characteristics than the pegylated form on the market. In addition, polymer encapsulation studies have been conducted with promising results, especially since it is a new alternative in the nanobiotechnology process. The project is underway with the development of processes of production, in GLP (good laboratory practice) and GMP (good manufacturing practice), of new L-asparaginases with higher characteristics and with important potential to be produced nationally and even for exportation.

ASPARAGINASE ENGINEERING IN THE OBTAINMENT OF BIOBETTERS OF THIS ANTITUMOR ENZYME.

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Asparaginase (ASNase), an enzyme biotechnologically produced in bacteria, is one of the most important compounds in the polychemotherapy to treat acute lymphoblastic leukemia (ALL) in children. There are only three options available as medicine: native enzyme from Erwinia chrysanthemi (ErA) or extracted from Escherichia coli (EcA) and formulated as native or PEGylated (PEG-EcA). However, these options yet present some problems in patients, such as to elicit hypersensitivity and allergenic reactions, neurotoxicity, and hyperammonemia. Aiming to avoid some of these problems, our research group has developed several different mutant proteoforms, expressed in bacteria and yeast, in periplasmic or secreted to extracellular

space; with improvement in specific activity, kinetic parameters, and stability; different oligomerization states, glycosylated or not, through engineering of genes from E. coli and E. chrysanthemi. We obtained mutants from E. coli ASNase more resistant to human proteases and less immunogenic. In relation to E. chrysanthemi enzyme, our mutants present higher asparaginase activity than the native form, with improved kcat. In addition, we obtained strains of Pichia pastoris that express glycosylated ASNases from bacteria. Our results suggest several biobetters options developed in this study.

PRODUCTION OF NOVEL GLYCOSYLATED L-ASPARAGINASE AS AN ALTERNATIVE AGAINST ACUTE LYMPHOBLASTIC LEUKEMIA.

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L-asparaginase (L-ASNase) is an important bacterial enzyme used as biopharmaceutical to treat acute lymphoblastic leukemia (ALL). Its use as medicine has important side effects such as pancreatitis, abnormalities in coagulation, hepatosplenomegaly, immunogenicity, among others. It has been counteracted by PEGylation; however, immunogenicity has been observed in PEG. Here we explore the production of recombinant L-ASNase from D. chrysamthemi glycosylated like-mammals in a Glycoswitch® Pichia pastoris strain as an alternative to PEGylation. In our results, the recombinant Erwinase occurred in three extracellular, glycosylated and biologically active variants; two of them tetramerics (Erw240) and (Erw160) with specific activity of 15.71 and 302.02 U mg-1 respectively; and one new monomeric version (Erw40) with 48.45 U mg-1. The lightweight tetramer and the monomer showed catalytic efficiency of 7.7 x 105 and 1.05 x 106 respectively. Mass spectrometry analysis of the more active tetrameric and monomeric versions showed mainly an oligosaccharide GlcNAc2Man7, bound to Asn170, which is part of a predicted immunogenic T-cell epitope. ELISA assay in vitro showed a significant reduction of antibody recognition in the Erw160, suggesting the oligosaccharide bound to L-ASNasa had a cloaking effect against antibodies. The new L-ASNase versions reported here could provide an alternative for the treatment of ALL.

MINISYMPOSIA

1. CARDIOVASCULAR AGING

PREVENTING PREMATURE ENDOTHELIAL CELL SENESCENCE: THE ROLE OF ANGIOTENSIN-(1-7).

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Vascular aging is a complex multifaceted process displaying functional and structural alterations that ultimately favour vascular disease and atherosclerosis. Endothelial cell senescence, which may be induced by a wide variety of extracellular stressors, is one of the major mechanisms contributing to vascular aging. The senescent endothelial cell acquire a phenotype characterized by growth arrest and the acquisition of a senescence-associated secretory phenotype (SASP), that promotes the release of pro-inflammatory mediators and the onset of sterile age-related inflammation. In this context, the identification of pharmacological tools to interfere with endothelial senescence may help retarding vascular aging and its complications. Angiotensin (Ang)-(1-7) is a heptapeptide belonging to the so-called protective arm of the renin-angiontesin system (RAS). Ang-(1-7) is a ligand for the G-protein-coupled receptor Mas. In the vascular system, Ang-(1-7) has been acknowledged as a physiological antagonist for angiotensin II (Ang II), since it displays vasorelaxant, anti-proliferative and anti-inflammatory actions, among other. Here, we tested the capacity of Ang-(1-7) to act as an anti-senescence molecule. In human cultured endothelial cells, Ang-(1-7) was capable to attenuate the pro-senescence actions driven by Ang II in terms of DNA damage, senescence-associated beta-galactosidase (SA-beta-gal) activity and SASP-related cytokine release. Importantly, Ang-(1-7) also attenuated the endothelial cell senescence induced by non-RAS stressors, such as the pro-inflammatory cytokine interleukin (IL)-1beta. These protective actions of Ang-(1-7) were mediated by Mas receptors since they were blunted by the Mas antagonist drug A779. Furthermore, we demonstrated that Ang-(1-7) exerted its anti-senescence actions by activating two cytoprotective systems, i.e., the Nrf2/heme-oxygenase axis and the anti-ageing protein klotho. Overall, the Ang-(1-7)/Mas receptor axis may a valuable pharmacological target to attenuate endothelial senescence and to delay vascular aging induced by a variety of stressors.

ROLE OF NLRP3 INFLAMMASOME IN VASCULAR DAMAGE INDUCED BY ADIPOKINES.

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We have demonstrated that these adipokines promotes vascular inflammation and endothelial dysfunction. Moreover, our data suggest that vascular deleterious effects evoked by visfatin/eNampt or sDPP4 may involve the activation of the NLRP3 (nucleotide-binding domain, leucine-rich-containing family, pyring domain-cotainin-3) inflammasome. Indeed, evidence from our laboratory demonstrates the activation of NLRP3 inflammasome by visfatin/eNampt, while the adipokine-evoked endothelial dysfunction is prevented by inhibiting its enzymatic activity with FK866, as well as by the antagonism of toll-like receptors-4 (TLR4) with CLI-095, the interference of NLRP3 inflammasome assembly with MCC950, or the IL-1 receptor blockade with anakinra. Therefore, we propose that visfatin/eNampt induces vascular damage by a TLR4-mediated pathway, leading to NLRP3-inflammasome activation and the paracrine production of IL-1beta. On the other hand, we have shown that sDPP4 induces vascular alterations by activating proteinase-activated receptors-2 (PAR-2) and upregulating thromboxane-A2 (TXA2) release. Moreover, the endothelial dysfunction evoked by sDPP4 is also dependent on its enzymatic activity, being attenuated by its inhibitors K579 and linagliptin, as well as by the specific PAR-2 antagonist GB83 and the TXA2 receptor blocker SQ-29,548. Interestingly, this pathway also leads to NLRP3 inflammasome activation, while the sDPP4-evoked endothelial damage is reduced by interfering NLRP3 inflammasome with MCC950. We conclude that vascular NLRP3 inflammasome activation can be a common pathway for different pro-inflammatory adipokines. Indeed, targeting NLRP3 inflammasome and some receptors linked to this pathway (TLR4, PAR-2, or IL-1) may represent therapeutic strategies to treat and/or prevent obesity-related vascular dysfunction.

CARDIAC FIBROBLAST ROLE ON INFLAMMATORY PROCESS: INTERACTION WITH IMMUNE CELLS.

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The abundance and strategic location of cardiac fibroblasts and also macrophages in cardiac tissue damage, suggest the possibility of a highly coordinated interaction between both cell types, in order to orchestrate the different stages of cardiac remodeling. In particular macrophage is able to adapt their phenotype and activity according to the cytokine milieu present in the local cardiac environment. This phenomenon, known as macrophage polarization, contributes to the accumulation of pro-inflammatory M1 macrophages during the onset of cardiac remodelling, while also explaining the high levels of anti-inflammatory/profibrotic M2 macrophages found in the later stages of cardiac repair. While the effects of macrophages on cardiac fibroblast activity have been extensively studied, the ability of cardiac fibroblasts to modulate macrophage behavior is less understood. LPS, and Heparan sulfate as pro-inflammatory stimulus, triggers on cardiac fibroblast ICAM-1 and VCAM-1 expression levels, which allow spleen mononuclear cells and neutrophils adhesion. LPS triggers high TNF-α/IL-10 ratio, whereas, TGF-β1 a profibrotic stimulus triggers an increase on ICAM-1 and VCAM-1 expression levels, but low TNF-α/IL-10 ratio. Consequently, cardiac fibroblast under LPS-treatment promote monocytes-macrophages M1 polarization. By contrast, cardiac fibroblast under TGF-β1 promote monocytes-macrophages M2 polarization. Our results demonstrate that cardiac fibroblasts interact with immune cells and contribute to monocyte recruitment and induce their differentiation to M1 or M2 macrophages.

2. NOVEL MOLECULAR PATHWAYS FOR SCHIZOPHRENIA

DYSREGULATION OF THE AMYLOID PRECURSOR PROTEIN AND IRON IN SCHIZOPHRENIA.

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Schizophrenia (Sz) is a debilitating mental illness that disrupts the functioning of the mind. Impairments in certain cognitive functions are core features of Sz, which are not addressed for existing drug targets and remain a crucial unmet therapeutic need. Our hypothesis is that schizophrenia is a complex disease resulting from a loss-of-function of key pathways that govern neurodevelopment, neurotransmission and synaptic connectivity. The Amyloid Precursor Protein (APP), which we have extensively investigated in relation to Alzheimer's disease, is a key regulator of brain structure and function. Our data indicate that iron is elevated in autopsy orbitofrontal cortex from individuals with Sz relative to age- and sex-matched controls. We hypothesize that these changes are mediated by the downregulation of APP, which also occurs in prefrontal cortex region of individuals with Sz. Our group have characterized the age-dependent accumulation of iron in the brain of global APP knockout mice. Remarkably, global APP KO display features of Sz, including agenesis of corpus callosum and increased seizure activity. Therefore, we propose that down regulation of APP function may represent a common lesion that leads to inappropriate neurotransmission, synaptic pruning and synaptic function that are involved in the clinical manifestation of Sz.

THE UBIQUITIN PROTEASOME SYSTEM IN SCHIZOPHRENIA.

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The aetiology of schizophrenia remains unknow. It has been linked to abnormalities in dopaminergic, glutamatergic, GABAergic and serotoninergic neurotransmission as well as signalling pathways critical for brain growth and maturation. A common consequence of these pathway abnormalities in schizophrenia is a loss in proteostasis of the key components of these extra/intracellular pathways. Proteostasis requires the control of protein synthesis, folding, conformational maintenance, protein-protein interaction, trafficking and degradation. The ubiquitin-proteasome system (UPS) is central to proteostasis, suggesting it likely plays a pivotal role in the onset and progression of schizophrenia. UPS is a master regulator of neural development and the maintenance of brain structure and function. It influences neurogenesis, synaptogenesis and neurotransmission by determining the localization, interaction and turnover of scaffolding, presynaptic and postsynaptic proteins. Although links between UPS dysfunction and neurodegenerative disorders have been known for some time, only recently have similar links emerged for neurodevelopmental disorders, such as schizophrenia. In this presentation, we will review the components of the UPS that are reported as dysregulated in schizophrenia by our group and others, and we will discuss specific molecular changes to these components that may explain the complex aetiology of this mental disorder as a syndrome.

DR. JORGE MARDONES RESTAT AWARD

1. USE OF NPSI-BCD COMPOSITE MICROPARTICLES FOR THE CONTROLLED RELEASE OF CAFFEIC ACID AND PINOCEMBRIN, TWO MAIN POLYPHENOLIC COMPOUNDS FOUND IN A CHILEAN PROPOLIS.

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Propolis is widely recognized for its various therapeutic properties, which are attributed to its rich composition in polyphenols. Polyphenols exhibit multiple biological properties, such as antioxidant, anti-inflammatory, anti-angiogenic, and others. Despite of its multiple benefits, oral administration of polyphenols results in bioavailability at the site of action. An alternative to face this problem is the use of biomaterials at nano-micro scale due to its high versatility as carriers and delivery

systems of various drugs and biomolecules. In that sense, the aim of this work is to determine if microparticles of nanoporous silicon conjugated with a beta cyclodextrin polymer to form the nPSi-βCD composite are available material for the controlled release of the two main polyphenols of Chilean propolis, caffeic acid and pinocembrin. Moreover, it was studied their cytocompatibility with HUVECs. Using different physicochemical techniques, it was demonstrated that nPSi-βCD microparticles successfully retained and controlled release caffeic acid and pinocembrin. Furthermore, nPSi-βCD microparticles presented cytocompatibility with HUVECs culture at concentrations of 0.25 mg/ml. These results suggest that nPSi-βCD microparticles can be safely used to improve the bioavailability of caffeic acid or pinocembrin –and eventually other polyphenols– in the target site, thus enhancing its therapeutic effect for the treatment of different diseases.

2. EARLY EFFECT OF A HIGH-FAT DIET ON PERIGONADAL AND HEPATIC FAT IN RATS.

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Obesity is a multifactorial disease of great impact in Chile and worldwide. Its causes are heterogeneous and there are no totally effective therapeutic interventions; therefore the study and advances in this subject are of great relevance. The dramatic increase in the levels of obesity in the population is related to a progressive change to sedentary lifestyles and excessive consumption of highly caloric foods, such as high-fat diets (HF). These factors lead to excessive accumulation of adipose tissue, and in the long term may result in ectopic fat storage. One way to study this disease and its comorbidities is the use of rodents fed with HF diet, as a model of diet-induced obesity. The aim of this work was to study the early effects of a HF diet on the accumulation of adipose tissue. For this, male Sprague-Dawley rats were fed a HF diet (62% of calories from fat) from postnatal day 30 for either 15, 30 or 60 days, and were compared to age-matched rats fed with a control diet (14% calories from fat). At the end of each treatment, perigonadal fat was weighed and total hepatic lipids were extracted. HF treated rats showed a significant body weight gain only until the end of the treatment compared to control diet rats. Regarding fat tissue, perigonadal fat was 77% higher in rat fed an HF diet and the percentage level of lipids in the liver increasing up to 8%. These results together suggest that a HF diet generates important physiological changes in the animal, producing in a short period a state of adiposity consistent with pre-obesity in rats.

3. INDOMETHACIN IMPAIRS POLYAMINE METABOLISM IN LUNG CANCER CELLS: A KRAS MUTATION-ASSOCIATED FEATURE?.

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Non-small cell lung cancer (NSCLC) is the most lethal and prevalent type of lung cancer. NSCLC patients carrying mutations in the Kirsten rat sarcoma viral oncogene homolog gene (KRAS) still lack targeted therapies. Also, the levels of polyamines (putrescine, spermidine, and spermine) are increased in cancer, playing a pivotal role in tumor proliferation. Indomethacin increases the levels of the polyamine-catabolic enzyme spermidine/spermine-N1-acetyltransferase (SSAT). Consequently, the aim of this study was to compare the effect of indomethacin in the polyamine metabolism of two NSCLC cell lines, with different KRAS mutation status. A549 and H1299 NSCLC cells (KRAS-mutated and wild-type, respectively) were exposed to indomethacin. Evaluations included SSAT expression and protein levels, and metabolic analysis of cells by CG-MS metabolomics. Moreover, the difference in polyamine synthesis enzymes among cell lines and the synergistic effect of indomethacin combined with inhibitors of these enzymes were investigated. Indomethacin increased the expression and levels of SSAT in both cell lines. In A549 cells, indomethacin significantly impairs polyamine metabolism. However, in H1299 cells, the impact of treatment on the polyamine pathway was non-significant. Evaluation of the levels of the polyamine synthesis enzymes showed that ornithine decarboxylase (ODC) is increased in A549 cells, whereas S-adenosylmethionine-decarboxylase (AMD1) and polyamine oxidase (PAOX), are increased in H1299

cells. Finally, indomethacin demonstrated a synergistic effect with the PAOX inhibitor MDL72527 in A549 cells, whereas in H1299 had a synergistic effect with the AMD1 inhibitors SAM486. Collectively, these results indicate that indomethacin alters polyamine metabolism in NSCLC cells and enhances the effect of polyamine synthesis inhibitors such as MDL72527 or SAM486. However, this effect varies depending on the basal metabolic fingerprint of each type of NSCLC cell. FONDECYT-1160807.

INCORPORATIONS

1. AMPK ACTIVATION ON CARDIAC FIBROBLASTS: ROLE IN AUTOPHAGY AND CELL PROLIFERATION INDUCED BY CATECHOLAMINES.

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Activation of the adrenergic system is commonly associated with cardiac fibrosis and remodeling, and cardiac fibroblasts are key players in these processes. Interestingly, adrenergic stimulation activates both, cardiac fibroblasts autophagy and cell proliferation, however, the underlying mechanisms have not been elucidated. In the present study, we assessed the effects of adrenergic stimulation on autophagy and cell proliferation in cultured adult rat cardiac fibroblasts, which were treated with beta-adrenergic agonists and antagonists. Autophagy was determined by electron microscopy, subcellular distribution and protein levels of LC3-II, and the signaling pathways involved in its activation after stimulation with catecholamines were evaluated by western blot. Our results suggest that AMPK plays a key role in the induction of autophagy, through the inhibition of mTOR activity. Indeed, the AMPK pharmacological inhibitor, compound c, prevents the autophagy induced by adrenergic agonists, acting downstream of AKT in the beta2-adrenergic receptor/AKT/mTOR pathway. AMPK activation was also necessary for ERK1/2 phosphorylation and cell proliferation. In addition, the increase in autophagy correlates with intracellular collagen degradation. In summary, here we show that beta2-adrenergic stimulation activates AMPK and this protein governs both processes, autophagy and proliferation in cardiac fibroblasts, therefore, a pharmacological modulation in this pathway could contribute to reducing the harmful effects of adrenergic stimulation in cardiac fibrosis.

2. THE EFFECT OF THE ALLOSTERIC INHIBITOR OF RIPK1 (NEC-1) ON OVARY FUNCTION: IMPORTANCE OF NECROPTOSIS IN FOLICULAR DEVELOPMENT.

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The ovarian follicle develops between proliferation and cell death process. Three types of cell death have been reported: apoptosis, phagocytosis and necrosis. A fourth type of cell death, necroptosis, has been recently associated to ovarian function. However, the physiological relevance of necroptosis or its involvement in follicular development it is not yet well understood. In the present work we will use pharmacological tools (allosteric inhibitor necrostatin-1), to study the role that Necroptosis would have in follicular development and ovarian function in vivo. We used two groups of animals: Sham and NEC-1. Adults rats were hemiovariectomized and implanted with a miniosmotic pump with NEC-1 (20 μM), for 28 days, or remains without drug administration (sham). At the end of the procedure, rats were euthanized and the ovaries and plasma were collected. The ovaries were fixed for morphometric analyses. Plasma levels of steroid hormones were measured by EIA. We found that necroptosis inhibition did not affect the number of secondary follicles, but increased total antral follicles by accumulating atretic antral follicles. The number of type III precystic follicles was increased while the cyst number did not change. Corpus luteum didn't change in number but decreased the new (bigger size) CL. An increase in testosterone plasma levels was found. In conclusion, NEC-1 treatment by blocking necroptosis in vivo, favored cyst formation and the permanence of old corpus luteum thus necroptosis could be involved in luteolysis and in the transition to follicular cyst in the ovary. In vivo models help to describe new pharmacological targets to regulate follicular development and hence fertility.

3. TLR4, BUT NEITHER DECTIN-1 NOR DECTIN-2, PARTICIPATES IN THE MOLLUSK HEMOCYANIN-INDUCED PROINFLAMMATORY EFFECTS IN ANTIGEN-PRESENTING CELLS FROM MAMMALS.

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Mollusk hemocyanins have biomedical uses as carriers/adjuvants and nonspecific immunostimulants with beneficial clinical outcomes. Hemocyanins have a multivalent nature as highly mannosylated antigens. We have shown that hemocyanins are internalized by antigenpresenting cells (APCs) through receptor-mediated endocytosis by innate immune receptors, such as mannose receptor (MR). However, the contribution of other pattern recognition receptors to the proinflammatory signaling pathway triggered by hemocyanins is unknown. Thus, we studied the roles of Dectin-1, Dectin-2, and Toll-like receptor 4 (TLR4) in the hemocyanin activation of murine APCs, both in dendritic cells (DCs) and macrophages, using hemocyanins from Megathura crenulata (KLH), Concholepas concholepas (CCH) and Fissurella latimarginata (FLH). The results showed that these hemocyanins bound to chimeric Dectin-1 and Dectin-2 receptors in vitro. However, hemocyanin-induced proinflammatory effects in APCs from Dectin-1 knock-out (KO) and Dectin-2 KO mice were independent of both receptors. Moreover, the phosphorylation of Syk kinase was not detected after hemocyanin stimulation. On the other hand, we confirmed a glycan-dependent binding of hemocyanins to chimeric TLR4 in vitro. Moreover, DCs from mice deficient for MyD88-adapter-like (Mal), were partially activated by FLH, suggesting a role of the TLR pathway in hemocyanin recognition to activate APCs. TLR4 role was confirmed through a decrease in IL-12p40 and IL-6 secretion induced by FLH when a TLR4 blocking antibody was used; a reduction was also observed in DCs from C3H/HeJ mice. Additionally, IL-6 secretion induced by FLH was abolished in macrophages deficient for TLR4. We further showed that KLH and FLH induced ERK1/2 phosphorylation. Our data showed the involvement of TLR4 in the hemocyaninmediated proinflammatory response in APCs, which could cooperate with MR in innate immune recognition of these glycoproteins.

4. DISCOVERY OF NOVEL TASK-3 POTASSIUM CHANNEL BLOCKERS.

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TASK-3 is a two-pore domain potassium (K2P) channel highly expressed in hippocampus, cerebellum, and cortex. TASK-3 has been identified as an oncogenic potassium channel and it is overexpressed in different cancer types. For this reason, the development of new TASK-3 blockers could influence the pharmacological treatment of cancer and several neurological conditions. In the present work, we search for novel TASK-3 blockers by using a virtual screening (VS) protocol that includes pharmacophore modeling, molecular docking, and free energy calculations (MM/GBSA). With this protocol, 19 potential TASK-3 blockers were identified. These molecules were tested in TASK-3 using patch clamp, and one blocker (DR16) was identified with an IC50 = 56.8 ± 3.9 μM. Using DR16 as scaffold we designed DR16.1, a novel TASK-3 inhibitor with an IC50 = 14.2 ± 3.4 μM. Our finding takes on greater relevance considering that not many inhibitory TASK-3 modulators have been reported in the scientific literature until today. These two novels TASK-3 channel inhibitors (DR16 and DR16.1) are the first found using a pharmacophore-based virtual screening and rational drug design protocol.

5. IMMUNOLOGICAL BASIS OF AUTISM: COGNITIVE EFFECTS OF AUTOANTIBODIES FROM AUTISTIC CHILDREN IN MEMORY AND LEARNING PROCESSES.

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Autism spectrum disorders (ASD) involve a range of complex neurodevelopmental disorders, characterized by social impairments, communication difficulties, and restricted, repetitive and stereotyped patterns of behavior. ASD exerts a significant physiological, emotional and financial burden on the families of the individual and society as a whole. Recently, beside the knowledge about genetic factors involved in this pathology, there is new evidence related to immunological causes of ASD. Therefore, it is of outmost importance to elucidate the molecular and physiological mechanisms of ASD pathology. Taking this into account, we hypothesized that ASD autoantibodies generates autoimmune-related cognitive impairment characteristic of ASD pathology. To achieve this aim, we used ex vivo experiments using hippocampal slices and a rat model where mothers were injected with ASD autoantibodies during pregnancy and/or breast milk period and the breeding was tested after that period using learning and memory test together with electrophysiological and immunohistochemical studies. Our results have shown that normal young rat hippocampal slices incubated with purified IgA autoantibodies from ASD patients and breeding rats from pregnant mothers injected with the same antibodies, impairs LTP as well as disrupts learning and memory. We also found that both LTP and learning and memory were significantly impaired in female but not male breeding rats and this alteration are correlated with the presence of ASD autoantibodies in hippocampal slices. These results demonstrate that ASD autoantibodies cross the transplacental barrier and also are ingested through breeding milk, crossing both intestinal and blood-brain barrier and impairs learning and memory in a sex-preference fashion. The pharmacological implicances of this research involve new mechanisms and possible therapeutical targets for ASD pathology

6. ANTI-STEROIDOGENIC EFFECT OF THE RFRP-3 NEUROPEPTIDE AND ITS PARTICIPATION IN THE FOLLICULAR DYNAMICS IN THE RAT.

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Ovarian function is highly regulated, either by hormones, autonomic nerves and paracrine signals produced by the same ovarian cells. Changes of these signals modified the normal functioning of the ovary. Gonadotrophin inhibitor hormone (GnIH) is a neuropeptide that block the GnRH secretion at hypothalamic level and therefore the gonadotropic axis regulating the ovary. The recently described receptor (GPR147 or NPFF1, homologous in mammals) and RFRP-3 (mammalian homologous peptide of GnIH) in the ovary open the possibility to suggest that the local presence of the peptide and its receptor could participates as regulator of ovarian function. We studied whether RFRP-3 and NPFF1 receptor are present in the rat's ovary and the local effect of this neuropeptide on hormone production and follicular dynamics. We determine the presence of RFRP-3 in the rat's ovary. RFRP-3 was mainly in the granulose cells of antral follicles and corpora lutea. Then, we studied the effect of 10 ng/mL RFRP-3 on the production of ovarian steroids ex vivo. RFRP-3 inhibited the hCG-induced ovarian progesterone and testosterone secretion. In order to know if the chronic presence peptide in the ovary modified the follicular development and its function, we designed a local chronic treatment in vivo with RFRP-3. After 4 weeks of treatment there was a decrease in serum testosterone and an increase in size and number of corpora lutea suggesting the appearance of new corpora lutea and hence increased ovulation. No changes appeared in secondary, antral, cyst or atretic follicles. Data indicate a local effect of RFRP-3 that positively affect ovarian steroidogenesis and follicular dynamics. This study opens new pharmacological targets, such as neuropeptides, to treat disorders in ovarian function.

ORAL COMMUNICATIONS

1. THE N-ACETYLCYSTEINE-INDUCED REDUCTION OF CHRONIC ALCOHOL COMSUMPTION IS ASSOCIATED TO THE ACTIVATION OF THE NRF2/ARE PATHWAY IN HIG-ALCOHOL-DRINKING UCHB RATS.

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Current evidence suggests that neuroinflammation and oxidative stress are associated to chronic alcohol consumption and relapse, suggesting that the modulation of oxidative stress induced by chronic alcohol drinking can be a therapeutic target in alcoholism. There is evidence that oxidative stress activates the Nrf2 (Nuclear factor erythroid 2-like), that translocates into nucleus where it promotes the transcription of antioxidant genes containing the Antioxidant-Response-Element (ARE) including hemoxigenase 1 (HO-1) and NAD(P)H dehydrogenase quinone 1 (NQO1). Previously we have demonstrated that N-acetylcysteine (NAC), a cysteine precursor, with antioxidant action, inhibits alcohol consumption, neuroinflammation and alcohol-induced oxidative stress in chronic drinking rats (UChB). However, the mechanism of the antioxidant action of N-acetylcysteine it is not clear. The present study determinates whether the NAC-reduction of chronic alcohol consumption is associated to the activation of the Nrf2 /ARE pathway in high-alcohol-drinking rats. Chronic alcohol drinking (61days) female UChB rats were administered for nine consecutive days either (i) NAC (100mg/kg/day, per os); (ii) NAC + all-trans-retinoic acid (ATRA, a Nrf2 pharmacological inhibitor (10 mg/kg/day ip); (iii) ATRA+ saline); (iv) Saline. After determining the rates of alcohol consumption, all groups were euthanized for hippocampal histological and Western blot analyses. It was found that (i) N-acetylcysteine inhibits chronic alcohol intake (ii) N-acetylcysteine induced Nrf2 nuclear translocation (ii) N-acetylcysteine-induced inhibition of chronic alcohol intake was prevented by ATRA a Nrf2 pharmacological inhibitor. In conclusion these results support the idea that Nrf2 activation is the mechanism by which NAC inhibited chronic alcohol consumption and relapse.

2. D-LACTATE INDUCES NEUTROPHIL EXTRACELLULAR TRAPS (NET) RELEASE BY DISTURBING CELLULAR METABOLISM.

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D-lactate is produced during acute ruminal acidosis (ARA), a well-known fermentative disorder in cattle. Recently, we demonstrated that heifers with ARA show aseptic neutrophilic synovitis, characterized by the presence of D-lactate, abundant neutrophils, NET, and metabolic disturbances in synovial fluid. It has been described that D-lactate entry is required to induce NET-release. Since D-lactate is slowly metabolized by mammalian cells, we hypothesized that D-lactate induces metabolic disturbances in neutrophils, and so could induce NET-release. Blood neutrophils isolated from 5 healthy heifers were treated with 5 mM D-lactate in vitro. First, we performed a GC-MS untargeted metabolomic analysis. D-lactate altered galactose metabolism, starch and sucrose metabolism, nucleotide sugars metabolism and glycolysis. Using JC-1 probe we observed by flow cytometry that D-lactate reduced $\Delta\psi m$. In addition, D-lactate favored the glycogen degradation, and increased glucose-1-P and glucose-6-P intracellular levels. Also, D-lactate increased the AKT and GSK-3β phosphorylation. The inhibition of theses pathways with LY294002 and CHIR99021, respectively, interfered the decrease of glycogen and NET release. Our results suggest that D-lactate induces NET release by disturbing cellular metabolic pathways, involved in glycogen degradation.

3. CHANGES PRODUCED BY PREBIOTIC FIBERS IN THE SURVIVAL OF LACTOBACILLUS CASEI, SUBSP CASEI DURING THE SHELF LIFE OF A NUTRACEUTICAL DAIRY DRINK.

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Introduction. The accelerated current lifestyle has generated important changes in the population's diet at global level. There are reports that indicate that the use of prebiotics and probiotics are important factors in the modulation and restoration of gastrointestinal tract microbiota (MTGI), since by decreasing intestinal permeability and gastrointestinal inflammation modify homeostasis of immunity related to glucose and lipid absorption, decreasing factors correlated with diseases such as obesity and overweight (Fukuda et al., 2014). The formulation and development of new nutraceutical foods fermented with probiotic microorganisms and added prebiotic fibers such as apple and potato could be a viable alternative in the treatment of emerging and high impact diseases such as those mentioned above (Mishra et al., 2019; Gibson et al., 2017). Objective. In the present work, the changes produced by prebiotic apple and potato fibers on the survival of Lactobacillus casei subsp. casei were evaluated, as well as the physicochemical changes produced during the shelf life of nutraceutical dairy drinks. Results. Dairy drinks fermented showed changes in the survival of L casei, and in the physical-chemical properties (viscosity, pH, production of lactic acid and syneresis over time (1 to 4 weeks). Conclusions. The results showed that although there was a decrease in the survival of L. casei in the two fibers under study (apple and potato), they preserved its probiotic properties (1x108 CFU / mL), while the physical and chemical changes observed over time (1-4 weeks) did not modify the sensory properties of fermented dairy drinks.

4. SYNTHESIS, CHARACTERIZATION, THEORETICAL STUDY AND IN VITRO EVALUATION OF BETA-LACTAMIC COMPOUNDS AND IMINES WITH POTENTIAL ANTIBACTERIAL ACTIVITY.

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One of the most serious problems worldwide is the resistance of the main pathogenic bacteria to the antibiotics used today. The evolution of the resistance seen in the light of the Darwinian and Lamarckian theories of adaptation gives rise to the understanding of the causes of resistance and if the causes are known, solutions can be proposed, otherwise, what will be achieved is to amplify the problem to such an extent that hospitals will become the repertoire of microbial infections resistant to any chemotherapeutic treatment. With the current biochemical knowledge, a rational design of antibiotics with in silico experiments of chloromonobactams was proposed from imines p-substituted with stereochemistry (E), which demonstrated that both sets of molecules comply with the Lipinski rule of 5, which offers a viable pharmacokinetics towards the organism. The synthesis of chloromonobactams was carried out in two phases. The first is the synthesis of p-substituted imines with (E) configuration; the second is a [2+2] Staudinger cycloaddition to obtain chloromonobactams.

The characterization of all the synthesized compounds was performed by physical tests (determination of Rf, melting point, and solubility tests), and spectroscopy (UV-visible and IR spectrophotometry, 1H and 13C NMR spectroscopy, and high-resolution mass spectrometry). The evaluation of the in vitro antibacterial activity was carried out by the disk diffusion method.The study strains were S. aureus sensitive to dicloxacillin, E.coli and P. aeruginosa sensitive to aztreonam. The results obtained so far show that the imines have antibacterial activity against the bacteria under study, with the p-iodo imine and the beta-lactam without substituents showing an activity similar to aztreonam on P. aeruginosa.

5. ROLE OF MITOCHONDRIAL METABOLISM IN OXIDATIVE RESPONSE AND NETS RELEASE INDUCED BY PAF IN BOVINE NEUTROPHILS.

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Neutrophils (PMN) constitute the main line of cellular defense in the innate immune response. Since they obtain energy primarily through glycolysis, it is assumed that they do not produce ATP by oxidative phosphorylation. However, mitochondrion of PMN maintains a transmembrane potential, which is normally associated with respiratory chain and oxidative phosphorylation for ATP synthesis. PMN were isolated from healthy heifers and stimulated in vitro with platelet activating factor (PAF), a key biochemical mediator in various inflammatory conditions. Incubation with PAF 100 nM increased mitochondrial transmembrane potential and mitochondrial reactive oxygen species (mtROS) production. While mtROS levels were reduced using rotenone 10 uM (mitochondrial complex I inhibitor), these were increased by oligomycin 10 uM (mitochondrial complex V inhibitor). PAF 100 nM also stimulated respiratory burst in PMN, which was reduced not only with 2-deoxy-D-glucose 2 mM (2-DG, glycolysis inhibitor), but also with rotenone 10 uM, oligomycin 10 uM and carbonylcyanide-3-chlorophenylhydrazone 5 nM (CCCP, oxidative phosphorylation uncoupler). Finally, PAF 1 uM induced neutrophils extracellular traps (NETs) release, which was reduced by 2-DG and CCCP, but increased by oligomycin. These results suggest that PAF triggers respiratory burst and NETs release through glycolysis and mitochondrial metabolism-dependent mechanisms.

Multitarget drug design for the treatment of Alzheimer's Disease.

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Neurodegenerative diseases (NDDs) are currently considered a worldwide pandemia with a prevalence of about 47 million people. It is estimated that, in 2050, two billion people will be over 60 years old, thus the number of people affected is expected to triple. Therefore, the search for effective drugs capable of controlling neuronal cell death is one of the great challenges of this century.
AD is associated with several neuronal abnormalities in energy metabolism such mitochondrial dysfunction, a decline in glucose uptake, dysfunction in Ca2+ homeostasis. It has been shown that oxidative damage occurs before the onset of significant Aβ plaque formation. For instance, the free radical theory of ageing implies progressive ROS cell damage with age, leading to enhanced mitochondrial DNA mutations, futile mitochondrial Ca2+ cycling with excess ATP consumption and ensuing mitochondrial dysfunction. On the other hand, it is now increasingly recognized that inflammation also strongly contributes to extensive oxidative stress found in AD brains. We therefore hypothesize that mitochondrial dysfunction could be the potential link between neuroinflammation and neurodegeneration. Another common characteristic is the interconnection between these pathways that causes feedback pathological loops that accelerates the advance of the disease. Therefore, their therapeutic approach must be directed to several pathological nodes, as the design of multitarget drugs capable of stopping different pathological pathways at the same time.

In this sense, the intrinsic cellular defense pathway, the Nrf2-ARE pathway, has been proposed as a therapeutic alternative for the development of effective drugs. Therefore, we are developing new multitarget compounds that combine the Nrf2 induction activity with other specific targets capable of reducing oxidative stress, neuroinflammation and the formation of protein aggregates, besides activating neuronal survival pathways that could be of potential therapeutic relevance to afford neuroprotection in Alzheimer's disease.

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6. THE CONSUMPTION OF A CALAFATE EXTRACT MODULATES THE ENERGY EXPENDITURE, FUNCTION AND MITOCHONDRIAL DYNAMICS OF BROWN ADIPOSE TISSUE OF OBESE MICE.

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Obesity is a public health problem of global concern. In its pathogenesis, the White Adipose Tissue has a crucial role. There is a mitochondrial dysfunction and lower oxidative capacity in adipocytes of obese individuals, with modifications in their morphology. In contrast, Brown Adipose Tissue (BAT) has a thermogenic function through UCP-1. A new approach proposes to increase energy expenditure through diet. Our objective in this work was to evaluate the effect of a calafate extract rich in polyphenols on mitochondrial energy, function and dynamics (fusion / fission) of obese mice. The analyzes were performed on adult C57BL / 6J male mice, which were subdivided (n = 10 each) into 4 dietary regimens / treatments: Control Diet (C), Control / Calafate (extract: 50 mg total polyphenols / kg weight; CC), High Fat Diet (HF) and High Fat Diet / Calafate (HFC). The mice were subjected to indirect calorimetry. Post-euthanasia was evaluated: gene and protein expression of UCP-1, PGC-1alpha, OPA1 (fusion), DRP1 (fission) (qPCR, western blot or immunofluorescence), mitochondrial Oxygen Consumption Rate (OCR) (XF24 Seahorse), HSP70 (amount of mitochondria) and mitochondrial activity (with MTO). The consumption of calafate extract produced an increase in energy expenditure and a decrease in respiratory quotient. The treatment presented differences at the level of mitochondrial function, with an increase in thermogenesis (UCP-1) a recovery of OCR, and a significant effect on the MTO / HSP70 ratio. It did not substantially modify the mitochondrial morphology. The consumption of a calafate extract rich in polyphenols increases energy expenditure and improves mitochondrial function in obese mice. Additional studies on mitochondrial dynamics are required to complement these hypothesis.

7. FROM HOMO SAPIENS TO HOMO TECHNOLOGICUS, BIOETHICAL CHALLENGE OF TRANSHUMANISM.

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Transhumanism, an empiricist thesis whose anthropology dispenses with metaphysics. It explains the human dynamism from functionalist neurobiologicism, which underlines the human to the functioning, of its neural connections, and that seeks its sustenance in scientific perfection. In the bioethical field it is founded is liberal utilitarianism. There are authors who argue that we are in the last stage of the development of homo sapiens, and in the era of homo technologicus, it has the possibility of continuing the evolution of the human species towards a superior, better and happier, using technology to its scope. Transhumanism raises many questions, among others. Has neurobiological physicalism been proven? Who tells me that the more perfect I am physically and psychically, that the more capacities I have, I will be happier? What is happiness? What does it mean to be better or more perfect, who determines it? We try a response in the moral and ontological field. Then there are issues of a practical nature when implementing the transhumanist plan: embryonic selection and eugenic elimination of embryos and fetuses with defects, problems derived from nanotechnology applied to the

brain and neuroethics, cryopreservation problems, use of drugs that change personality, resource distribution problems, etc.
This study aims to address the ethical and anthropological challenge that underlies transhumanism.

8. SYNTHESIS AND EVALUATION OF INDOLYL-BENZAMIDO-PIPERAZINES AS POTENTIAL MULTI-TARGET-DIRECTED LIGANDS IN ALZHEIMER´S DISEASE.

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Alzheimer's disease (AD) is a chronic, progressive and fatal neurodegenerative disorder affecting cognition, behavior, and function, being one of the most common causes of mental deterioration in elderly people: Around 50-60% of the overall dementias correspond to AD. World Health Organization estimates that about 46.8 million of people worldwide currently suffer from AD, thus becoming a major public health concern as the world's population ages. (World Alzheimer's report, 2018). Development of Multi-Target Directed Ligands (MTDLs) has emerged as a promising approach for targeting complex etiology of Alzheimer's disease (AD). Following these approach, and given our interest in the search and development of novel drugs displaying affinity acting as promiscuous ligands. In the present work, a novel series of indolylpropylpiperazinyl piperazinebenzamides were synthesized and biologically evaluated as multifunctional ligands in the following targets: acetylcholinesterase, SERT, and beta-amyloid peptides The synthesis involved connection between Piperazine benzamides with N-Boc substituted piperazine derivative. Boc cleavage and further coupling with indolylpropyl tosylates was achieved in two step-one pot reaction, obtaining the final compounds with good to excellents overall yields. Finally, the obtained compounds were evaluated in its capabilities for AChE inhibition, SERT- affinity, β-amyloid inhibition, and cell toxicity (viability) with very promising results.

9. DEVELOPMENT OF A RECOMBINANT VACCINE CANDIDATE AGAINST HANTAVIRUS.

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Andes virus is the main causative agent of Hantavirus cardiopulmonary syndrome (HCPS) in South America. There are currently no vaccines or treatments against Andes virus. However, there are several evidences suggesting that antibodies against Andes virus envelope glycoproteins may be enough to confer full protection against HCPS. The main goal of the present work was to develop a vaccine candidate against Hantavirus, based on the surface glycoproteins Gn and Gc. With this purpose, the sequence encoding the extracellular domains of both antigens was introduced into the methylotrophic yeast Pichia pastoris. After induction with methanol, the recombinant antigens accumulated intracellularly as insoluble aggregates. After cell disruption, the recombinant antigens were solubilized and purified by metal-ion affinity chromatography. The immunogenicity of both antigens was determined in immunization assays in both mice and Syrian hamsters. In both species it was possible to detect the presence of specific antibodies against Gn and Gc. Part of these antibodies showed neutralizing activity. The results obtained to date suggest that the Gn and Gc antigens from Andes virus, produced in P. pastoris, have the potential to become the first commercial vaccine against HCPS.

10. ADHERENCE TO ANTIHYPERTENSIVE PHARMACOLOGICAL TREATMENT IN ELDERLY PEOPLE FROM HUALPEN SUBMITTED TO A TRANSMEDIAL PSYCHOEDUCATIONAL PROGRAM.

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In Chile, the number of elderly people has steadily increased, with 20% of the population projected by 2025. This change associated with a sedentary lifestyle is linked with an increased prevalence in chronic pathologies such as Hypertension. The national prevalence is 27.6%. The lack of pharmacological adherence constitutes one of the main problems in the control of the disease, considering that only approximately 50% of the patients adhere properly. Given this problem, a Transmedial Psychoeducational Program (PST) was developed to support primary care treatment, focused on knowledge of the disease, as well as promoting the benefits of pharmacological treatment and a healthy lifestyle.
The PST was evaluated in two CESFAM in the commune of Hualpén with three levels of intervention: Group A (n = 104) through a mobile application "AFAM-Health", Group B (n = 97) using video capsules and Group C (n = 98) as control.
The average age of the 299 elderly was 72 ± 7.6 years, 83.7% have as their source of income the retirement salary, 88.6% lives, accompanied, 74.3% attend their health checks alone and 68.6% of them walk. After one year of intervention, group A was significantly more adherents (Morisky-Green test) over time with values of 52, 73, 64 and 64% of adherents at the beginning, third, sixth and twelfth month, respectively. Group A had 4.5 ± 1.8 medications / day, 43.4% corresponded to antihypertensives, Losartan is the main one.
From these results, it is determined that older adults who use the "AFAM-Health" App as a support increase pharmacological adherence unlike those who only receive the traditional primary care treatment.

POSTERS

1. POSTER RETIRADO"

2. SEARCH FOR ANALOGS OF M554 AND M890 FOR INTERACTION WITH THE GBΓ DIMER IN REGIONS INVOLVED IN THE REGULATION OF THE GLYCINE RECEPTOR.

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Ethanol is the drug with highest consumption levels, with effects at different levels of the Central Nervous System. Accute consumption at high levels can induce coma and death. This molecule modulats the activity of Glycine receptor (GlyR), a ligand gated ion channel that belong to the cys-loop subfamily of ion channels. Recently it has been identified that Gbg protein as a modulator of the channel interacting with the cytoplasmic domain and potentiating the activity of the channel. With determined structure of Gbg and GlyR cytoplasmic domain, it has been able to identify chemical entities to inhibit the etanol effects. Initially, peptides were designed and then peptidomimetic small molecules were developed, like M554 and M890. These molecules interact with Gbg and inhibit etanol effects in vitro and in vivo. After that new molecules were designed applying bioisosteric changes in the original molecules M554 and M890. Through bioinformatic technics like docking , molecular dynamics and free energy calculations (MM-GBSA), it has been identified the derivatives (R,S)-M554_3, (S)-M554_13, (R)-M554_13, M890_4 y M890_5 which interact in the Gbg hotspot surface with conserved aminoacids. Finally, cytotoxicity assays were performed in HEK cells determining that the molecules were not toxic for cells.

3. BIOGUIDED ISOLATION OF SECONDARY METABOLITES PRESENT IN THE MEDICINAL SPECIES CALDCLUVIA PANICULATA (CUNONIACEAE) WITH INHIBITORY ACTIVITY IN VITRO ON THE ENZYME A-GLUCOSIDASE.

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Diabetes mellitus (DM) is a metabolic disease characterized by an increase in blood sugar levels. According to the World Health Organization, 422 million adults worldwide had DM by 2014. There are studies based on ethnobotanical knowledge that support the use of medicinal plants with hypoglycemic activity, ascribing their activity to the presence of phenolic compounds. Mapuche medicine suggests the consumption of "Tiaca" (Caldcluvia paniculata) as a hypoglycemic treatment. The objective of the research was to evaluate in vitro the inhibitory activity of secondary metabolites present in C. paniculata on a-glucosidase. For this purpose, the plant material was defatted and macerated in a hydroalcoholic solution for 7 days. The hydroalcoholic extracts were dried, resuspended (MeOH:H2O - 70:30) and partitioned by liquid extraction, with solvents of increasing polarity. The activity of the partitions was determined by inhibition on a-glucosidase: Aqueous solutions of the dry extract were prepared at different concentrations (1-100 ug/mL), using p-nitrophenyl-(1,4)-a-D-glucopyranoside as a substrate and acarbose as positive inhibition control. The positive control of inhibition on the enzyme (acarbose) presented an IC50 of 1288 ug/mL. Ethyl acetate partition presented the lowest IC50, reaching values of 13.6 and 14.5 ug/mL for leaf and stem respectively. The most active partition was fractionated by column chromatography using silica gel and eluted with solvents of increasing polarity. The 15 groups obtained were evaluated for their inhibitory capacity on a-glucosidase. Group G. 9 presented better inhibition with an IC50 of 20.2 ug/mL. The chromatographic profiles of leaves and stems were analyzed by HPLC, observing similar profiles and the presumptive presence of phenolic compounds. The results obtained are conclusive regarding the hypoglycemic property of C. paniculata.

4. DETECTION AND IDENTIFICATION OF ANTIBACTERIAL COMPOUNDS IN LIQUID FERMENTATIONS OF FUNGUS STEREUM SP. BY HPTLC-BIOASSAY-MS.

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Basidiomycetes belonging to higher fungi, offer an exciting field to obtain new structures with high potential for medical applications. Higher fungi have an important advantage as producers of bioactive secondary metabolites: they release them to liquid media. Then, the objective of this work was to detect and identify compounds with antibacterial activity in liquid fermentations of fungus Stereum sp. Pure mycelial cultures were produced from impressions of spores of fruiting bodies, which were then cultured in YMG medium (glucose, malt extract, yeast extract and agar) previously sterilized. For liquid fermentation, the following was performed: small sections (15-10) of 5 mm diameter plug were cut under sterile conditions and transferred to an Erlenmeyer flask containing liquid YMG medium. The flasks were incubated at 20-22 °C on a Shaker orbital shaker with constant shaking. The cultures were stopped when abundant mycelia were observed, the glucose source was emptied, and the pH was about 7. The liquid culture was filtered to separate the broth and the mycelium. Bioactive compounds were extracted with ethyl acetate from culture media. The total extract was concentrated to dryness in a rotary evaporator (45 °C), weighed and stored at 4 °C. The extract dissolved in methanol was seeded on HPTLC plates silica gel 60 F254. Separation was performed using the mixture of toluene-ethyl acetate (3.15 : 1.85 v/v) as a mobile phase. The extract was seeded in triplicate by dividing the HPLTC plate into three sections: the first section was used for the bioassay (direct bioautography), the second section for the chemical derivatization and

the third section for the mass spectrometry analysis (MS). After chromatography, the first section was dried and a buffer solution was atomized. The plate was immersed in Bacillus subtilis bacteria suspension and incubated at 37 °C for 2 hours. Subsequently, the plate was atomized with a solution of methylthiazolidiphenyl-tetrazolium bromide (MTT), incubated at 37 °C for 30 min and finally dried completely on a heating plate at 50 °C for 5 min. A zone of inhibition was detected on the HPTLC plate as a colorless zone/band on a purple background. Using the third section of the plate (dried previously), this bioactive/inhibitory zone was directly eluted by means of the TLC-MS interface coupled to the electrospray ionization source (ESI) of a triple quadrupole mass spectrometer. Full scan mass spectra (m/z 100-1000) were recorded in positive (ESI+) ionization mode. The bioactive compound tentatively corresponds to Himanimide C.

5. EFFECTS OF ARSENIC (AS) EXPOSURE ON BLOOD-BRAIN BARRIER AND COLONIC PERMEABILITY IN HEALTHY YOUNG RATS.

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Arsenic (As) is a toxic metalloid, which has become a health burden worldwide. Growing evidence indicates that As has harmful effects on the central nervous system (CNS), as this metalloid crosses the blood-brain barrier (BBB). On the other hand, there is little evidence suggesting that loss of intestinal permeability might affect BBB permeability. The aim of this study is to evaluate if oral exposure to As affects intestinal and BBB permeability, as this pollutant might have an impact on what now is known as the brain-gut axis. Methods: Female Sprague–Dawley rats (PND35) where given 10 ppm of $NaAsO_2$ in the drinking water for 24h (n=5), and compared to control rats (n=6). At 24h the following samples were collected: brain, colon, lung, stool and liver tissues. Each sample was lyophilized and then microwave digested in order to determinate total As concentration by HPLC-HG-AFS. Additionally, colonic permeability to FITC-dextran 4.4kDa (FD4) was evaluated ex vivo for 120 and 180 min by everted gut sac technique. Results: Gut permeability to FD4 is increased in animals exposed for 24h to 10 ppm of $NaAsO_2$ in comparison to controls. In addition, the metalloid concentration was higher in every studied tissue of exposed rats, in comparison to controls. In the brain, As was found in hypothalamus and cerebral cortex. This data suggest that As is able to cross the BBB and increases gut permeability in the rat, an effect that might lead to alterations in BBB. In conclusion, a toxic pollutant such as As might cause alterations in the brain-gut axis, effects which gives a novel approach in the study of As toxicity.

6. TWO CONSERVED ALPHA-HELICES IN CORTICOTROPHIN RELEASING FACTOR BINDING PROTEIN CONTAINING A HYDROPHOBIC PATCH DETERMINES ITS SORTING TO THE REGULATED SECRETORY PATHWAY.

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Corticotrophin releasing factor binding protein, (CRF-BP) is a 37 kDa glycoprotein that binds CRF with high affinity. CRF-BP in the periphery controls CRF levels in plasma during pregnancy. In the central nervous system, CRF-BP facilitates the traffic of CRFR2alfa acting as an escort protein. Previously, it has been shown that CRF-BP enters the regulated secretory pathway (VSR). However, the sorting signal(s) are presently unknown. We decided to determine the sorting signal(s) of CRF-BP to VSR. We used NPS @, an in silico secondary structure prediction tool and PEPWHEEL to draw predicted alpha helixes and the in silico modeling of CRF-BP protein structure. Additionally, we did studies of sorting of chimeras containing the putative sorting signals in PC12 cells over-expressing the selected chimeras. In silico analysis and modeling of CRF-BP protein structure showed the presence of three alpha-helix domains, (50-74), (128-149), (229-251). The alpha-helixes domain (50-74) and (229-251) in CRF-BP is highly conserved among different mammalian species and has a hydrophobic patch characteristic of other sorting domains

to the VSR. The results show that the alpha-helix domain (50-74)-CRFBP is capable of restore the sorting of a chimeric variant of proCART precursor, without its sorting domain to the VSR. Furthermore, the presence of the alpha-helix domain (50-74)-CRF-BP in the chimeric variant of proCART allowed its secretion triggered by a depolarizing stimulus. Our results show that the preserved alpha-helix domain (50-74)-CRF-BP, present in the amino terminal of CRF-BP, is responsible for its destination to the VSR. Further studies are needed to evaluate if the other alpha-helix domains also play a role in the sorting of CRF-BP to the VSR.

7. TTAGP 1.0: A COMPUTATIONAL TOOL FOR THE SPECIFIC PREDICTION OF TUMOR T CELL PEPTIDES.

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Nowadays, cancer is considered a global pandemic and millions of people die every year because this disease remains a challenge for the world scientific community. Even with the efforts made to combat it, there is a growing need to discover and design new drugs and vaccines. Among these alternatives, antitumor peptides are a promising therapeutic solution to reduce the incidence of deaths caused by cancer. In the present study, we developed TTAgP, an accurate bioinformatic tool that uses the random forest algorithm for antitumor peptide predictions, which are presented in the context of MHC class I. The predictive model of TTAgP was trained and validated based on several features of 922 peptides. During the model validation we achieved sensitivity = 0.89, specificity = 0.92, accuracy = 0.90 and the Matthews correlation coefficient = 0.79 performance measures, which are indicative of a robust model. TTAgP is a fast, accurate and intuitive software focused on the prediction of tumor T cell antigens.

8. NGF INCREASED CHOLINE ACETYL TRANSFERASE AND THE VESICULAR ACETYLCHOLINE TRANSPORTER EXPRESSION IN RAT OVARY EX VIVO.

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The ovary is an endocrine organ which is regulated by hormonal and neural signals. It is well known that noradrenaline controls ovarian steroidogenesis and folliculogenesis. Its source comes from sympathetic neurons that innervate the ovary. In addition, evidence suggests that acetylcholine enhances follicular development and its intraovarian production would be in granulosa cells which express choline acetyltransferase (ChAT), vesicular acetylcholine transporter (VAChT) for storage and acetylcholinesterase. Besides, cholinergic muscarinic receptors (M1, M3, M5) are expressed in ovarian follicles. However, it is not known how this intraovarian cholinergic system is regulated. In vitro studies had shown that human granulose cells incubated with neuronal growth factor (NGF), a neurotrophin produced by ovary, increased ChAT. The objective of the present work was to determine if NGF enhances acetylcholine production in the rat ovary. 26 days old rats ovaries were incubated with 100 ng/mL NGF during 3 and 24 hrs. 7 days old rats were treated with 50 mg/Kg guanethidine during 3 weeks to induce a chronic endogenous NGF increment and, after 3 months, ovaries were obtained. We measured mRNA levels by qRT-PCR, acetylcholine levels by fluorometric assay, NGF and noradrenaline by ELISA kit, and NGF by western blot. After 3 hrs, NGF produced an increment in ChAT and VAChT mRNA levels, but a decrease in acetylcholine in medium. After 24 hrs, we found a modest but constant increase in acetylcholine production. Guanethidine treatment didn't induce an endogenous NGF increment despite that noradrenaline levels decreased. Altogether these data suggest that NGF regulates intraovarian acetylcholine production and storage ex vivo. Further research is needed to elucidate if a longer time of NGF stimulation is needed to better visualize the neurotransmitter.

9. AMPHETAMINE AND TEMPOL MODULATE EXTRACELLULAR CONCENTRATION OF DOPAMINE AND THE PHOSPHORYLATION LEVEL OF THE DOPAMINE TRANSPORTER.

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Amphetamine (AMPH) is a highly reinforcing, widely abused stimulant drug

that increases dopamine extracellular levels in the mesocorticolimbic system, in neurons projecting from the Ventral Tegmental Area to Nucleus Accumbens and Prefrontal Cortex. AMPH-stimulated efflux of dopamine through the dopamine transporter (DAT) only happens if DAT is previously phosphorylated. Some kinases that act on DAT are PKC, ERK and PKA and their activity is modulated by different signaling pathways. The increase in the production of reactive oxygen species (ROS) after the intake of AMPH, besides producing brain damage, can modulate the action of these kinases. Scavenging ROS may be a way to avoid the toxic effects of oxidative stress induced by AMPH. Tempol is an antioxidant that has neuroprotective activity, diminishing the presence of oxidative markers in the brain. It has been shown that Tempol interferes with the development of behavioral sensitization induced by cocaine. We evaluated the effect of Tempol and AMPH in the phosphorylation level of DAT using rat Nucleus Accumbens synaptosomes that were stimulated with AMPH, Tempol and Tempol before AMPH. We also measured the concentration of dopamine in the medium where the synaptosomes were incubated by microdialysis and electrochemical detection. Western Blot analysis showed that AMPH increased the phosphorylation of DAT and that the presence of Tempol avoided this increase. The extracellular concentration of dopamine in the presence of AMPH alone was significantly increased. No changes were observed in the presence of Tempol alone. Interestingly, AMPH in the presence of Tempol induced a lower increase in dopamine concentration. Taken together, these findings suggest a role of ROS in the mechanism by which AMPH increases dopamine extracellular levels in Nucleus Accumbens.

10. COLCHICINE COMPETITIVELY ANTAGONIZES THE ALPHA 3 SUBUNIT OF THE GLYCINE RECEPTORS.

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Glycine receptors (GlyRs) are anion-selective neurotransmitter-gated ion channels, member of the pentameric Ligand Gated Ion Channels (pLGICs) family. GlyRs are essential players in the physiology of the central nervous system and impairment of its function underlie many neurological diseases, including epilepsy, autism, chronic pain, anxiety and schizophrenia among others. The function of GlyRs containing the alpha1 or alpha2 subunits, can be competitively inhibited by colchicine independently of microtubule depolymerization. Interestingly, a recent report showed that colchicine binds directly to the GlyRs containing the alpha3 subunit, suggesting that the alpha3 GlyRs mediated the suppression of the inflammatory pain exerted by colchicine. However, the functional effects on the alpha3 GlyRs function elicited by colchicine are still undefined. Using electrophysiological techniques and molecular docking simulations, here we show that colchicine is an inhibitor of the alpha3 GlyR function. Colchicine, elicited concentration-dependent inhibitory effects on alpha3 GlyRs at micromolar range. Single-channel recordings show that the colchicine inhibition is associated with a decrease in the open probability of the ion channel. Molecular docking assays suggest that colchicine preferentially bound to the orthosteric site in an agonist-free, closed state of the ion channel. Our results thus define the pharmacological modulation of colchicine on alpha3 GlyRs.

11. CHARACTERIZATION OF THE NEUTROPHIL/LYMPHOCYTE RATIO IN A SAMPLE OF PATIENTS WITH RHEUMATOID ARTHRITIS.

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Introduction: Rheumatoid arthritis (RA) is a systemic disease, still unknown etiology and autoimmune character characterized by chronic inflammation in the synovial membrane. The activity of the disease in RA is determined with DAS28 index, which allows evaluating the efficacy of the drug therapy administered. Monitoring the evolution of the disease activity is essential to avoid disability in long term. The increase in the neutrophil/lymphocyte ratio (NLR) has been described as a parameter of inflammation associated with a predominance of neutrophils and a decrease in lymphocytes. This NLR relationship has proven to be a good predictor of inflammation

in chronic diseases such as diabetes and cancer. Objective: To characterize the NLR relationship in patients with RA. Methods: 11 AR patients and 11 controls of similar ages and same sex were recruited and signed an informed consent approved by Ethical Scientific Committee. A blood sample was performed on a Sysmex XS-1000i device. The absolute values of neutrophils were divided by lymphocytes. The values of DAS28 and erythrocyte sedimentation rate (ESR) were analyzed. Statistical tests were used for variables. Results: The NLR was higher in RA patients, 2.72±0.68 versus controls with 1.53±0.38 p<0,0001. The classification of disease activity according to DAS28 shown that NLR was 3.69 ± 0.25 for high activity, 2.49 ± 0.29 for moderate and 2.23 ± 0.32 for low and the coefficient of HSV-NLR correlation was 0.34 and DAS28-NLR was 0.815. Conclusion: The NLR was higher in AR patients than in the control group. It is shown that NLR correlates positively with DAS28 and HSV. In addition, the NLR was higher for patients with high disease activity, compared to moderate and low.

12. GPER ANTAGONISM OF THE POTENT ANTHOCYANIN-INDUCED VASODILATION AND NO PRODUCTION IN A VASCULAR BED AND ISOLATED ENDOTHELIAL CELLS.

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Anthocyanins are colored water-soluble flavonoids present in red-bluish berries, fruits and vegetables. Flavonoids conserve structural similarities with other molecules such as steroids. Genistein is recognized as a natural phytoestrogen with potent estrogenic activity. Considering the potent vasodilation elicited by the glycosylated or anthocyanin aglycones is strictly endothelium-dependent, we proposed that the anthocyanin-induced vasodilation is mediated by the estrogen receptor coupled to a trimeric G protein (GPER) or estrogen receptor alpha (ERa), both associated to a rapid, non-genomic mechanism. We evaluated the vascular response induced by the anthocyanin delphinidin and its 3-O-glucoside derivative (D3G) in pre-contracted mesenteric vascular beds in the presence or absence of 1μM-G36, a purported GPER receptor antagonist, or 1μM fulvestrant a recognized ER α/β antagonist. Also, we quantified the NO production elicited by anthocyanidins by chemiluminescence. Both and D3G elicit concentration-dependent vasodilation as potent as acetylcholine used as a standard control and 10 to 100 times more potent than 17-beta-estradiol (E2) and genistein respectively. The anthocyanins activity is dependent on the endothelium and eNOS enzymatic activity (97-98%, p <0.001). G36 significantly reduced the anthocyanins vasodilation (p <0.01) such as G-1, GPER agonist (p <0.001). However, the anthocyanins response was not blocked by fulvestrant, which decreased E2 activity by 28%. The perfusion of the mesenteric vascular bed with 100 nM or 1 μM of delphinidin and D3G, or its application to endothelial cells culture, increased NO production compared to the controls. We conclude that anthocyanins, glycosylated or not, induce a potent vasodilator response mediated by NO production that may be linked to GPER activation at the vascular level. In addition, the anthocyanin mechanism is rapid and non-genomic in nature.

13. MOLECULAR CHARACTERIZATION OF HEAD AND NECK SQUAMOUS CELL CARCINOMA (HNSCC) 3D MODEL AND METASTATIC CAPACITY EFFECTS OF OLD DRUGS WITH NEW ANTITUMOR ACTIVITY.

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Head and neck squamous cell carcinoma (HNSCC) has an incidence in worldwide of more than 350000 people and the mortality associated with this cancer is 50%. Although the treatment for these patients generally consists of surgical, pharmacological and radiation therapies, survival at 5 years is only 53%. One of the reasons for high mortality and low survival is due to the presence within tumor mass a subset of cells called cancer stem cells (CSCs), which represent the most tumorigenic subpopulation. This population grows like spheroids and in its center has hypoxic cells that are highly resistant to chemotherapies. Here, the goal was to obtain in vitro spheroid CSC from HNSCC (HNCSCs) and determine expression protein level involving on metabolism, hypoxia, autophagy, and antitumor target, in addition to assess the effects of Itraconazole and hydroxychloroquine on invasion capacity on spheroid cultures. Spheroid formed from

Cal27 and HEp-2 cell lines using culture selecting conditions and protein expression levels was determine by immunoblotting. Spheroid was treated with Itraconazole and hydroxychloroquine and then seeded over matrigel to Boyden chamber 3D invasion assays. Successful formation of spheroid from the Cal27 and Hep-2 cell lines, where needed 4500 and 3500 cells respectively to obtain sizes greater than 300 µm. Spheroids were also subjected to hypoxic conditions with oxygen concentrations below 5%, VDAC, PDK1, Hexokinase II, Hif-1 and LC3B protein expression levels were different between cell line and monolayer or spheroid cultures. HNCSCs exposure to Itraconazole and hydroxychloroquine modulate invasive capacities, compared with control conditions. Here we showed that protein expression patterns are different between monolayer and spheroid cultures and the effects of two different drugs on metastatic capacity of HNCSCs.

14. MESENCHYMAL STEM CELL SECRETOMES (MSCSS) ADMINISTRATION IMPROVES BEHAVIOURAL DEVELOPMENT, MOTOR AND COGNITIVE IMPAIRMENTS INDUCED BY PERINATAL ASPHYXIA.

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Perinatal asphyxia (PA) induces deficits in neurological reflexes, development, motor coordination, emotional behaviour and cognition. At present, no treatment significantly attenuates or prevents these sequelae. Mesenchymal stem cells (MSCs) have been proposed as a therapeutic tool for several CNS diseases, since MSCs display remarkable antioxidant, anti-inflammatory and repairing features (neurogenesis, synaptogenesis, myelination). MSCs exert paracrine effects by secreting a combination of nano-vesicles and soluble factors (referred to as secretomes). Preconditioning MSCs with pro-inflammatory cytokines (TNFalfa plus INFgamma) or hypoxia-like environment (deferoxamine) improves their effectiveness. The aim of this study was to determine whether intranasal administration of secretome derived from preconditioned human MSCs prevents the (i) behavioural development, (ii) motor and (iii) cognitive disabilities resulting from PA. PA was induced by immersing foetuses-containing uterine horns into a water bath at 37 °C for 21 min. Two hours after birth and at postnatal day (P)7 MSCSs (6 ug/16 ul, obtained from 1x10□6 preconditioned-MSCs) or 16 ul of vehicle were administered intranasally to asphyxia-exposed or control rats. Neurobehavioral development was evaluated by monitoring the righting reflex (at P1, P4, P7 and P14); negative geotaxis (P7, P14 and P21), and cliff aversion (P7, P14 and P21). Locomotor activity (P7) and motor coordination (P60) were evaluated by open field and rotarod, respectively. Anxiety (P30), by open field and novel object recognition memory (P30). All the PA induced effects were positively affected by MSCSs treatment, including improvements in: (i) the remarkable developmental delay in the performance of righting and cliff aversion reflexes; (ii) the decrease in locomotor activity and deficits in motor coordination and balance; (iii) an increase in anxiety and novel object recognition memory deficits.

15. INHIBITORY EFFECT OF COUMARINS DERIVATIVES ON THE VIABILITY OF HELICOBACTER PYLORI ATCC 43504 AND RECOMBINANT CARBONIC ANHYDRASE ACTIVITY.

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Helicobacter pylori (Hp) is a Gram negative pathogen that affects more than 50% of the world population, and it is responsible for different gastric pathologies. Since increasing

antibiotics resistance cause serious failure in Hp eradication therapy, new pharmacological targets need to be identified to affect the viability of this bacteria. Carbonic anhydrase (CA, EC 4.2.1.1) is an interesting new Hp target since it is involved in neutralizing the acid pH of the human stomach cooperatively with urease. It has been described that specific coumarin derivatives can inhibit CA. Besides, several coumarins exhibit a strong antibacterial activity. Objective: to clone and characterize carbonic anhydrase from the highly aggressive strain Hp ATCC 43504 and to determine the coumarins derivatives effects on both, the recombinant protein and Hp viability. Methodology: a-CA was cloned, expressed in cell line HEK 293 and purified. Recombinant α-CA was characterized by esterase activity and protonography. We determined the inhibitory effect of coumarins derivatives against recombinant α-CA by esterase activity and on Hp to determine its MIC and MBC. Results: A 50 kDa functional recombinant Hp a-CA was cloned that forms a dimer structure in solution. The recombinant Hp a-CA it is closely related to F78 strain, with a 99.1% of identity. Esterase activity was determined, complemented by protonography analysis, and kinetic parameters. Overall, six coumarins showed inhibitory activity against Hp, with MIC ranging from 125 ug/mL to 15 ug/mL, and two coumarins were inhibitors of the recombinant protein. Conclusion: The characterization of this highly aggressive Hp CA strain and the determination of inhibitory effects of coumarins, makes them a potential candidate for Hp therapy and eradication

16. IDENTIFICATION OF POTENTIAL CANDIDATE GENES RELATED TO HYPOXIA INDUCIBLE FACTOR 1 ALPHA (HIF-1 ALPHA) INVOLVED IN PERINATAL ASPHYXIA IN RATS.

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Perinatal asphyxia (PA) is characterized by interruption of oxygen bioavailability at birth. Hypoxia implies HIF-1 alpha (HIF-1a) activation, a key sentinel protein, which, upon translocation to the nucleus, binds to response elements (HREs), promoting transcription of several genes. Potential HIF-1a activated genes were identified in the rat genome following hypoxic conditions, by extracting promoter sequences of Rattus Norvegicus from the UCSC database Genome Browser. 8762 genes with the HIF-1a binding sequence (5`-RCGTG-3`) were identified using the "R" software. These genes were introduced to the Gen Ontology platform for performing an enrichment analysis, selecting the following processes linked to PA: (i) Hypoxia (865 genes); (ii) Glucose Metabolism (330 genes); (iii) Neurogenesis (1243 genes); (iv) Apoptosis (814 genes); (v) Angiogenesis (165 genes), and (vi) Regulation of Gene Expression (2076 genes). 865 hypoxia-associated genes were further selected and compared with experimental data by ChIP-Seq, with 772 and 98 genes from human and zebrafish genomes, respectively, identifying 79 genes for the three species. The 8762 genes were then analysed by the Kyoto Encyclopedia of Genes and Genomes (KEGG) platform, selecting the HIF-1 pathway, identifying 47 genes. The 79 genes filtered for human, zebrafish and rat were compared with the 47 genes obtained by the KEGG platform, yielding 12 genes. Finally, 12 genes were compared with 47 genes referred by the literature to be associated to PA, identifying 5 genes: (i) Bcl2; (ii) Hif-1a; (iii) Ldha; (iv) Pdk1, and (v) Vegfa. The pharmacological inhibition of HIF-1a to establish the gene expression levels of candidate genes in an in vivo model of PA is studied, providing a proof-of-principle for the participation of HIF 1a on the regulation of gene expression following PA.

17. DEVELOPMENT AND CHARACTERIZATION OF A NANOPARTICULATE SYSTEM FOR NASAL ADMINISTRATION OF CURCUMIN DERIVATIVE.

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In the present work, the nanoparticles (NPs) of chitosan were elaborated and characterized by means of the ionic gelation method, using for this the magnetic stirring and as a crosslinking agent sodium tripolyphosphate (TPP) and / or a derivative of thiamine. The optimization of the NPs of the chitosan the response has been carried out by means of a central composite design, in which the different parameters have been evaluated as

the relationship between the mass between the chitosan/derivative of thiamine/TPP, different speeds and times of agitation, pH of the solutions and order of addition of the components, with respect to the average diameter, polydispersity and zeta potential of the NPs. The physical characteristic of the best formulation gave spherical shapes, with an average diameter of 136,12 ± 3,60 nm, a polidispersion index of 0,42 ± 0,02 and a zeta potential of +31,06 ± 0,97 mV for white NPs. The NPs loaded with curcumin have a stability of 90 days at 5 °C ± 3 °C and a stability of 6 days at 25 °C ± 2 °C with 60 % ± 5 % relative humidity. A cell viability study was carried out, using the THP-1 cell line, the results indicated that the NPs with a concentration of 11 µg/mL curcumin equivalents, the viability is higher than 80 %. Finally, the encapsulation efficiency of curcumin in the final NPs formulation was 24,4 ± 4,48 %, with a load of 455 ± 0,06 ug%, with a size, PDI and zeta potential of 218,3 nm, 0,134 and +31,9 mV, respectively. Keywords: nanoparticles, chitosan, curcumin, cell viability.

18. RESOLVIN D1 INCREASES COLLAGEN-1 SYNTHESIS ON RAT CARDIAC MYOFIBROBLAST THROUGH ALX/FPR2 RECEPTOR ACTIVATION.

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Background: Cardiac fibroblast (CF) to cardiac myofibroblast (CMF) differentiation is mainly mediated by TGF-b1 released from immune cells by angiotensin II (Ang II) effect, or in vitro conditions due to mechanical stress. CMF are able to produce extracellular matrix proteins, mostly collagen, in the scar formation proccess. Also, in the CF-to-CMF process it has been demonstrated the upregulation of Kinin-B1 and AT1R receptor, among others. However, the presence of Resolvin D1 (RvD1) receptor, ALX/FPR2, has not been elucidated. RvD1 is an anti-inflammatory lipidic mediator that regulates matrix proteins like a-SMA and collagen in various cell types, yet there is not evidence of this effect on CMF. Purpose: To demonstrate that the CF-to-CMF differentiation increases ALX/FPR2 protein levels, and consequently, ALX/FPR2 activation by RvD1 enhances collagen-1 synthesis. Methods: Secondary culture of adult rat CF was starved for 24 hours, stimulated with TGF-b1 (10 ng/mL) for 72 hours on fetal bovine serum (FBS) 1% to induce CMF differentiation and then treated with RvD1 at different intervals in presence and absence of PD98059, LY294002 (ERK ½ and AKT inhibitors) and WRW4 (ALX/FPR2 antagonist). The proteins levels of p-EKR ½, p-AKT and collagen-1 were measured by western blot. Results: After CMF differentiation, it was found an increase of ALX expression. On CMF, RvD1 activated the ERK ½-AKT pathways and enhanced the expression of collagen-1. These effects were blocked by PD98059, LY294002 and WRW4. On the other hand, RvD1 did not decrease α-SMA protein levels, which suggests that it does not reverse CMF differentiation. Conclusions: Our results suggest that the CF-to-CMF differentiation increases the protein levels of ALX/FPR2, and that RvD1 enhances collagen-1 synthesis through ERK ½ and AKT pathways.

19. PROCYANIDINS-RICH EXTRACT NANOEMULSIONS O/W AS A POTENTIAL ANTITUMORAL TOOL: EVALUATION IN MELANOMA CELL LINE.

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During the last 10 years, the incidence of melanoma in Chile has incremented 27.2%. Approximately 80% of all skin cancer-related deaths are attributed to melanoma and long-term survival of patients is only 5%. This type of cancer is considered a multifactorial disease related to environmental interaction and genetic susceptibility that trigger a constitutive activation of several signaling pathways, promoting proliferation and survival of tumoral cells. Current therapies cause a considerable number of side effects, still representing a global human health issue. Recently, the use of polyphenols has been

reported for both prevention and treatment of melanoma. Some evidence demonstrates that different extracts of polyphenols (grape, pomegranate, among others) are high potential candidates to treat various types of cancer. Of note, the main limitations of these molecules are the low bioavailability and the necessity of a high concentration dose to cause a biological effect. The aim of this study was to utilize the nanotechnology to encapsulate procyanidins and to evaluate its antitumoral activity in B16F10 melanoma cell line. To achieve the proposed objective, an avocado peel procyanidin-rich extract was used and cellular viability, proliferation and migration in B16F10 cells were evaluated. Nanoemulsions were elaborated by solvent evaporation and were in a nanometric range of 170 ± 2 nm and showed low polydispersity (between 0.1 and 0.2). The nanoformulations showed negative zeta potential (−44 ± 4 mV), realizing a stable system. The administration of procyanidin-rich extract nanovehicles was more efficient in reducing cellular viability, proliferation and migration compared to free extract. Altogether, our results suggest that encapsulation of procyanidins significantly improves its effect on melanoma therapy.

20. MATERNAL HIGH-FAT DIET CONSUMPTION DURING PREGNANCY AND LACTATION IMPAIRS THE INHIBITORY SYNAPTIC TRANSMISSION OF CA1 REGION OF HIPPOCAMPUS OF THE YOUNG OFFSPRING.

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Before birth and early in life the brain development is acutely sensitive to its environment. Experimental and clinical data indicate that maternal obesity can predispose the offspring to suffer metabolic and neuronal alterations. Most studies have focused on the functional relationship between maternal obesity and hypothalamus alterations. However, the impact of maternal nutrition on other brain areas remains elusive. Recently, it has suggested that fetal exposure to maternal obesity causes decreased neurogenesis and impaired hippocampal learning. The hippocampus is important for learning and memory, and its development is sensitive to the metabolic environment in utero. In a model of maternal obesity induced by a high-fat diet (HFD, 60Kcal in fat) consumption we study whether this adverse prenatal environment impairs the hippocampal synaptic transmission. In offspring mice, during adolescence, using electrophysiological recordings in the CA1 area of mice hippocampus, we observe that maternal HFD consumption increases the frequency and amplitude of Inhibitory postsynaptic currents. This increase in inhibition level onto pyramidal neurons could have important consequences in the excitation/inhibition balance, being able to modify the hippocampal cognitive function in juvenile offspring mice.

21. PHARMACOKINETIC VARIABILITY IN PATIENTS WITH KIDNEY TRANSPLANTATION, TREATED WITH CYCLOSPORINE.

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Introduction: Cyclosporine (CsA) is an immunosuppressive drug, used to prevent organ rejection in transplant patients. However, this drug is characterized by having a narrow therapeutic range and high inter and intra-individual pharmacokinetic variation. There are some genetics variants involved in pharmacokinetic variability, both at the level of cyclosporine absorption and metabolization. However, genetic variants of relevant impact are not yet identified. Objective: to evaluate the genetic variants involved in the pharmacokinetics of cyclosporine and to establish an association

between the pharmacogenetic profile, and the safety and efficacy of the treatment during the first three months after transplantation. Methodology: One hundred and seven patients (107) with kidney transplants from San Juan de Dios Hospital (Project No. 028-13) were retrospectively included and were genotyped for the genetic variants CYP3A4 *1B rs2740574, CYP3A4*22 rs25599367, CYP3A5*3 rs776746, POR*28 rs1057868, MDR1 3435 rs1045642, MDR1 2677 rs2032582 and MDR1 1236 rs1128503. Genotypic frequency were associated with blood concentrations of cyclosporine (CsA), creatinine value and blood pressure within the first three months post-transplant. Results: Heterozygous patients for CYP3A4*1B had a lower creatinine value from the second post-transplant week. In relation to cyclosporine levels, an increase was observed, in patients with at least one altered allele for CYP3A5*3 from the second post-transplant week. Conclusions: The results show that genetic variants can account for variations in the pharmacokinetic parameters of cyclosporine, which affect the efficacy and safety of cyclosporine treatment. It is expected that, based on the results found, a genetic predictive panel of response to CsA will be setted-up to be used before transplantation.

22. ANTIFUNGAL AND ANTIBIOFILM EFFECT OF OREGANUM VULGARE ESSENTIAL OIL AGAINST CANDIDA ALBICANS AND NON-ALBICANS.

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Oral candidiasis is the most common fungal infection in humans. The most frequent causative agent isolated is Candida albicans (C. albicans), but the number of resistant strains such as Candida krusei (C. krusei), Candida tropicalis (C.tropicalis) and Candida Glabrata (C.glabrata) has increased. It is treated locally with miconazole and nystatin; however, patients present high recurrence rates due to the formation of biofilms. Biofilms are biological communities adhered to a surface (oral mucosa, dental prostheses) with high resistance to antifungals, which has driven the search for natural alternative therapies, such as essential oils. Oregano essential oil (OE-O) is effective and a therapeutic option on bacterial biofilms, but its activity on fungal biofilms is unknown. Methods. The minimum inhibitory concentration (MIC) was determined on reference strains for C.Albicans (fluconazole sensitive and resistant) and non-albicans strains (C.tropicalis, C.krusei and C.glabrata). The antibiofilm effect was evaluated by: a) morphogenesis inhibition assay: an inoculum was incubated for 5 hours at 37°C in the presence of the OE-O MIC, then the percentage of filamentous cells was counted using a Neubauer chamber; and b) inhibition of biofilm adhesion: an inoculum was incubated in a 96-well plate in the presence of the CIM of OE-O for 4 hours at 37°C, the adhered biofilm was stained with crystal-violet and absorbance was measured in microplate reader. Fluconazole and nystatin were used as controls. Results. AE-O significantly inhibited both biofilm adhesion and morphogenesis of the strains studied compared to controls. Conclusion. These results indicate that OE-O has significant antibiofilm activity in both C. albicans and non-albicans strains.

23. EFFECTS OF MODAFINIL ADMINISTRATION ON SOCIAL PLAY BEHAVIOUR AND DOPAMINE TRANSMISSION IN JUVENILE RATS.

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Modafinil (MOD) is a stimulant used to enhance wakefulness and vigilance. The mechanism of action of MOD has not been completely elucidated but a blockage of dopamine (DA) and norepinephrine transporters has been observed. Some clinical trials are testing MOD for the treatment of attentional deficit disorder (ADD). In view of a reported over diagnostic of ADD, evaluating the effects of MOD in healthy individuals is important. Herein, we evaluate the effects of MOD on social play behaviour (SPB) and

DA extracellular levels in juvenile rats. 35 juvenile male Sprague-Dawley rats were treated from PND25 with MOD (75 mg/kg i.p.) or vehicle for 14 days. Locomotor and social exploration were tested 24 hours after de last injection. Nucleus Accumbens (NAc) and ventral tegmental area (VTA) were dissected to measure DA content by HPLC coupled to electrochemical detection. We observed a decrease in the "pinning events" (responses to play behaviour) and tendency to decrease in the pouncing latency (the events of solicitation to play) in the MOD group. Also, there was a decrease in DOPAC/DA and DOPAC content in VTA. No differences in social exploration time and DA content were observed. SPB is fundamental to establish social and cognitive development in highly social animals like rats and humans. These preliminary results show that MOD could affect SPB by altering the rewarding effects of socialization. Importantly, DA levels are almost the same in both NAc and VTA, although there is a tendency for lower levels in MOD group. More studies are needed to unravel the effects of stimulants, specially on young population, over important social skills like playing, social interactions and memory

24. HIGH-FAT DIET EXPOSURE INCREASES THE EXPRESSION OF KEY PROTEINS IN DOPAMINERGIC NEUROTRANSMISSION OF RAT LATERAL SEPTUM.

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Obesity is a global pandemic that must be studied from many points of view, such as social, preclinical, clinical, economic, etc. At the level of the central nervous system, there are several structures involved in the control of food intake, being one of the most important to promote the feeding the lateral hypothalamus (LH). The main brain area that controls the neural activity of LH is the lateral septum (LS), which it sends GABAergic projections towards LH, controlling feeding behavior. In addition, LH projects glutamatergic/orexinergic neurons to the ventral tegmental area (VTA), which it sends dopaminergic projections to the nucleus accumbens and LS. This circuit is very important for the intake of rewarding foods, but it is not known the effects of chronic exposure to high-fat diet (HFD) on LS neurotransmission. For this work we used 2 groups of male Sprague-dawley rats exposed from weaning to postnatal day 60 (PND 60) to chow diet (control) and HFD. At PND 60 the animals were euthanized and the LS was microdissected to measure by western blot key proteins involved in LS dopaminergic neurotransmission. Our results demonstrate that exposure to HFD results in a significant weight gain at the end of the experimental period together with an increase in retroperitoneal fat levels. In LS the chronic exposure to HFD resulted in an increase in the expression of the type 2 dopamine receptor (D2) and the dopamine transporter (DAT) compared to control rats. These results suggest that these proteins functionally reduce the dopaminergic tone in LS, which would affect their inhibitory control over LH activity. However, the implication of these results will be evaluated in subsequent experiments.

25. BOLDINE PREVENTS TGF-B1-INDUCED DIFFERENTIATION OF CARDIAC FIBROBLASTS BY INHIBITING FOXO1.

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Diabetes and myocardial infarction promote the development of cardiac fibrosis. Normally, cardiac fibroblasts (CF) are responsible for the synthesis and maintenance of extracellular matrix components (ECM), whereas in pathological conditions CFs differentiate into cardiac myofibroblasts, generating an imbalance in the secretion of the ECM proteins. TGF-beta1 plays a crucial role in the development of cardiac fibrosis by regulating the expression of FoxO1, a transcription factor that is involved in functions such as apoptosis, oxidative stress and cell differentiation. On the other hand, boldine, a natural alkaloid, has been shown to exert antifibrotic effects in experimental models of diabetes. Therefore, this work attempted to demonstrate the antifibrotic effect of boldine in a model

of cardiac fibrosis in vitro. To respond to this hypothesis, the differentiation of adult Sprague-Dawley rats CF by TGF-beta1 was used as in vitro model of cardiac fibrosis. The differentiation of CF was determined by the expression of alpha-SMA and CTGF, through western blot (WB) and immunocytochemistry (ICQ). On the other hand, the activation of FoxO1 was evaluated by analyzing phospho-FoxO1 (WB) and nuclear location of FoxO1 (ICQ). AS1842856 a FoxO1 activity inhibitor was used to evaluate the role of FoxO1. TGF-beta1 10ng/ml increased the expression of alpha-SMA and CTGF at 48h, which was corroborated by an ICQ against alpha-SMA. In addition, TGF-beta1 increased the activity of FoxO1, which was determined by decreased phosphorylation of FoxO1 and increased nuclear localization, whereas inhibition of FoxO1 prevented the differentiation of CF induced by TGF-beta1. Finally, boldine 50uM and 100uM abolished the differentiation of CF and the activation of FoxO1 induced by TGF-beta1. Collectively ours results suggest that boldine prevents TGF-beta1-induced CF differentiation by inhibiting FoxO1.

26. CHEMICAL-BIOLOGICAL CHARACTERIZATION OF ANTIOXIDANT PIGMENTS PURIFIED FROM EXTRACTS AND FRACTIONS OF PENICILLIUM MURCIANUM AND ITS POTENTIAL COSMETIC APPLICATIONS.

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In order to find new sources of active metabolites as functional ingredients for the cosmetic industry, the idea of studying filamentous fungi that produce natural pigments arises. These compounds meet the requirements of having low toxicity, adverse effects, and generation of polluting waste or expensive raw material. Therefore, due to their nature and structural diversity they could have great potential for cosmetic use, for example, as hair dyes. To characterize the fungal pigments and evaluate their antioxidant effect, Penicillium murcianum species was selected. This fungus is an eco-type found in Chilean native forests, which was cultivated under conditions previously optimized for the production of brown-yellow pigments. To facilitate the chemical-biological analysis, the extract obtained from the culture broth was fractionated by Centrifugal Partition Chromatography (CPC) with a phase system of ethyl acetate: butanol: water. The above allowed to purify several fractions, which were evaluated for their antioxidant activity in the DPPH assay. These fractions recorded an inhibition close to 90% and an IC50 value of 4.64 mg / mL for the crude extract. In the case of the purified fractions, two were selected with the highest antioxidant activity corresponding to an IC50 of 1.17 mg / mL and 0.708 mg / mL, which were subsequently analyzed by HPLC / PAD / MS. Our preliminary results revealed the presence of azafilones such as monashexenone, monankarin and monaphilol and the anthraquinoids sterigmatocystin, endocrocin and flavokermesic acid, which would be responsible for the yellow pigmentation of the extract. These compounds have bibliographic antecedents related to antioxidant and antimicrobial activities, among others, which highlights a great opportunity for future research and applications for these metabolites of P. murcianum.

27. IN VITRO PROPAGATION OF RODOPHIALA PRATENSIS AND ITS TOXICITY IN VITRO ON EPITHELIAL CELLS OF GASTRIC ADENOCARCINOMA (AGS).

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Amaryllidaceae is a family of bulbous plants, producers of alkaloids which are biogenetically related and exhibit high pharmacological activity. Lycorine and homolycorine alkaloids have been studied as potent antitumor agents. However, the study of these molecules is difficult due to the low availability and production in the plant in the wild, so the objective of this study is by in vitro propagation to obtain biomass of R. pratensis in an efficient and sustainable way, to identify the type of Alkaloids that are produced and assess their cytotoxic potential on tumor cells. Methodology: Rhodophiala prantesis bulbs, were sterilized and cut into twin-scales, to sow them in Murashige-Skoog growth medium, supplemented with sucrose and different combinations of naphthalenacetic acid and 6-benzylaminopurine, the alkaloid analysis was performed by CG-MS. AGS cells were cultured in DMEM medium supplemented with SBF (10%), antibiotic (1%). The assay was performed in 96-well plate using resazurin at 6 and 24 hours after exposure of the alkaloid extract. R. pranthesis callus were obtained in in vitro culture in semi-solid medium. In addition, 25 alkaloids were identified in the bulb's alkaloid extract which decreased the viability of AGS cells at 6 and 24 hours of exposure. Conclusion: Hormonal combinations were evaluated for the production of callus of R. pratensis, in addition the alkaloids have cytotoxic activity on AGS as a function of exposure time.

28. SYNERGISTIC EFFECT OF GENTISIC AND GALLATE DERIVATIVES WITH STANDARD THERAPY FOR COLORECTAL CANCER CELLS.

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Colorectal cancer (CRC) is the third leading cause of cancer death in the world. The standard drugs currently used for the treatment of CRC are 5-fluouracil (5-FU), oxaliplatin and irinotecan. These chemotherapeutic agents are effective in the early stages of the disease, presenting high toxicity and many side effects. Therefore, new therapies directed to the metastatic cells of the CRC are urgently needed, with high pharmacological efficacy, reducing the side effects and treatment costs. In recent years, neoplastic mitochondria are an attractive pharmacological target for cancer therapy, since they have higher mitochondria potential. In our laboratory, gallic acid derivatives linked to an aliphatic chain of ten carbons associated with triphenylphosphonium (TPP+C10), a lipophilic cationic molecule that induces the uncoupling of the electron transport chain was synthesized and evaluated in CRC cells as well as gentysic derivative (GA-TPP+C10). The objective of this study is to evaluate the synergistic effect of the compounds, GA-TPP+C10 and TPP+C10 in combination with conventional drugs for the treatment of CRC, 5-FU and oxaliplatin, using the COLO 205 metastatic human CRC cell line. Through the MTT assay, the cytotoxicity of the combinations was evaluated after 48 h of treatment and by flow cytometry the synergy of the combinations inducing apoptosis was evaluated. The observed results showed that bothTPP+C10 and GA-TPP+C10 are synergistic with low concentrations of 5-FU and Oxaliplatin, inducing greater apoptosis of the colorectal cells. In conclusion, the results suggest that these new compounds could synergize the effects of conventional therapy against colorectal cancer.

29. CHANGES IN DOPAMINE RECEPTOR TYPE 2 EXPRESSION IN PREFRONTAL CORTEX INDUCED BY EARLY-LIFE INTESTINAL DYSBIOSIS.

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Intestinal microbiota has been shown to modulate central nervous system function. For instance, a reduction in the gut's microbiota richness and diversity through the use of antibiotics, sensitizes mice to drug

seeking behaviors. Furthermore, this behavior is driven by changes in dopamine receptor expression within the mesocorticolimbic system, which strongly supports the relevance of the microbiota-gut-brain axis in the development of addictive behaviors. In most animals, early-life gut colonization by microbial symbionts occur begins at birth, with bacteria coming from the mother, and is a process that happens in parallel with early stages of brain development. Therefore, changes in maternal gut microbiota richness and diversity would impact on the development of neuropsychiatric diseases later in life, including addiction. To test this, we administered pregnant Spraque-Dawley dams a cocktail of oral wide-spectrum antibiotics (neomicyn, bacitracin, vancomycin and pimaricin) from embryonic day 18 till post-natal day (PND) 7, and then evaluated dopamine receptor 2 (D2) expression in the prefrontal cortex (Pfx) of female and male offspring at PND60, and compared with the offspring of pregnant dams given saline. The results show that Pfx D2 expression in female offspring of antibiotic exposed dams is reduced (although not significant) when compared to age matched controls, but no differences were observed in the male offspring. These results suggest that a reduction in the diversity and richness of gut microbes during early-life provokes changes in PFx D2 expression in females, but not males, and furthermore, this changes might impact the female's drugs seeking behaviors.

30. EARLY-LIFE EXPOSURE TO ORAL WIDE-SPECTRUM NON-ABSORVABLE ANTIBIOTICS AND THEIR EFFECTS ON DOPAMINE RECEPTOR 2 EXPRESSION IN THE SUBSTANTIA NIGRA OF SPRAGUE-DAWLEY RATS.

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Microbial gut colonization begins at birth, where the transfer of intestinal microbes from mother to infant is key in early-life development of gut, immune and brain physiology. We have previously shown that exposing pregnant Sprague-Dawley dams to an oral cocktail of wide-spectrum non absorbable antibiotics (neomycin, bacitracin, vancomycin all three at a dose of 100 mg/kg and pimaricin at 5microg/kg) from embryonic day 18 until post-natal day (PND) 7, lowers intestinal microbial richness and diversity in the male offspring at PND35. Additionally, early-life exposure to antibiotics lowers dopamine receptor 1 (D1) expression in key areas of the mesocorticolimbic circuit of male offspring when compared to age matched controls that were not exposed to antibiotics perinatally. This suggest that early-life exposure to antibiotics, which affects gut microbial ecology, impacts on dopaminergic circuits related to reward. However, another question was raised: what happens in substantia nigra (SN), another major source of dopamine that is also involved in reward and motor function. Thus, we evaluated D2 expression in the SN of males (PND35) from Sprague-Dawley dams exposed to the aforementioned cocktail of antibiotics, and compared it with control rats. Immunohistochemical analysis revealed that early-life exposure to antibiotics does not affect D2 expression in comparison to control rats. This result suggest that reduction of microbial diversity and richness in early life, affects specifically the mesocorticolimbic circuit, with no effects on the D2 expression in the SN. Such specific effect further suggests that within the microbiota-gut-brain communication, there are very specific pathways that may in part underlie the basis of neuropsychiatric disorders, such as addiction.

31. MECHANICAL STIMULATION INCREASES EXTRACELULLAR ATP AND NO SECRETION TO THE MEDIA OF CELL CULTURES; PHYSIOLOGICAL IMPLICATIONS.

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As a requirement of many protocols it is common to add drugs to cell cultures to investigate cell processes in the presence of a determined pharmacological agent. However, if this procedure is not well controlled by appropriate standards, it may cause additional experimental variability. Our working hypothesis proposed that

pipetting/agitation of the culture medium causes extracellular ATP secretion, which in the case of endothelial cells is also related to NO secretion. Endothelial cells of the rat mesentery, fibroblasts, and oocytes of Xenopus Laevis were used. In endothelial cell cultures, 100 uL of Tyrode buffer is gently applied; extracellular fluid sample is collected to measure ATP and NO production. ATP and metabolites are quantified as fluorescent ethene purines; separated and quantified by HPLC procedures. NO was determined by a fluorescent probe as the [DAF-NO] complex. Buffer application increases medium ATP from 117 ± 75.7 to 317 ± 25 pmoles ATP/ mg protein ($p < 0.001$); the signal peaked by one minute, thereafter, ATP decays. This stimulus also rapidly and significantly increases NO production ($p < 0.0001$). This increase is blunted by 150 µM L-NAME, an eNOS inhibitor, and by apyrase (4 U/mL), suggesting the participation of extracellular ATP. The agitation of fibroblast culture medium in culture increases 4-fold ATP secretion, a transient effect that decreased in minutes. Likewise, Xenopus oocytes agitation by a variable inclination agitator increased 3.8 times extracellular ATP ($p < 0.01$). In conclusion, different mechanical stimuli secrete extracellular ATP in different cell types, suggesting that cells respond to chemical and sensory stimuli by increasing nucleotide secretion. The nucleotide surge may cause unexpected variations in the final cellular response due to indirect or direct purinoceptor activity.

32. MODIFIED SYMPATHETIC NERVOUS TERMINALS AND ENDOTELIAL CELLS FROM THE MESENTERIAL ARTERIAL BED BY STREPTOZOTOCIN-INDUCED DIABETES.

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Respiratory, intestinal and / or vascular epithelial cells modify the activity of its adjacent smooth muscle layer. We studied whether the prostatic vas deferens epithelium modulate the motor response induced by electrical stimulation by releasing an epithelial messenger. To this aim, the isometric muscular contraction of the prostatic segment of the rat vas deferens intact or mechanically denuded of its epithelium was recorded using a force displacement transducer. The tissues were placed in a super fusion bath with Tyrode buffer at 37°C, 95% O2-5% CO2. Muscle tension was recorded by a Grass polygraph coupled to a transducer; contractions were induced by electrical field depolarization or with chemical stimuli. The muscular contraction induced by the exogenous application of 100 uM norepinephrine decreased following epithelium removal (1.5±0.2g versus 0.8±0.1g, n =10, $p<0.0038$), but not those elicited by ATP or KCl. Exogenous ATP, at concentrations that do not induce contractions (1-100 uM), reduced the motor effect induced by electric trains of 0.15 Hz. Epithelial removal reduced this ATP effect ($p < 0.05$). ATP reduces muscle contraction induced by 4 Hz trains, in the phasic component, epithelial removal causes a greater inhibitory effect of ATP ($p < 0.05$). The inhibition of NO synthesis with 100 uM N-omega-nitro-l-arginine does not modify the inhibitory effect of ATP on the electrical stimulus. In contrast, indomethacin increases the inhibitory effect of ATP on 0.15 Hz in the absence of epithelium, and on 4 Hz, both in the presence and absence of epithelium. Altogether, present results suggest that a non-identified arachidonic acid derivative, but not NO, sensitizes the motor response of the duct, demonstrating that the epithelium participates and modulates its motor activity.

33. CHANGES IN THE EXTRACELLULAR LEVELS OF DOPAMINE IN NUCLEUS ACCUMBENS OF ADULT RATS NEONATALLY EXPOSED TO SEX HORMONES: STUDIES OF BRAIN MICRODIALYSIS USING METHYLPHENIDATE.

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Sex hormones produce several effects in reproductive and non-reproductive tissues. In this sense, brain expresses sex hormones receptors in cortical and limbic areas. Nigrostriatal and mesocorticolimbic

pathways are modulated by sex hormones, affecting the expression of key dopaminergic proteins in adult animals. However, few studies have been focused in long-term effects produced by early exposure to sex hormones. Last time, our lab has been shown some neurochemical and behavioral changes produced by neonatal administration of sex hormones in brain dopamine areas such as higher levels of dopamine (DA), expression of tyrosine hydroxylase and addictive-like behaviors induced by morphine. Last time, we published that adult rats exposed during first hours of postnatal life to sex hormones had a lower locomotor activity induced by methylphenidate (MPD) than control rats and this effect was associated with a lower expression of the dopamine transporter (DAT) in nucleus accumbens (NAcc). Therefore, the aim of this work was studied the basal and stimulate (MPD: 5 mg/kg) extracellular levels of DA, Glutamate and GABA in NAcc of adult rats exposed during the first hours of postnatal life to estradiol valerate (EV: 0.1 mg/50µL). Our results showed a lower NAcc DA release induced by MPD in EV rats compared to control rats. In control rats we observed in NAcc a reduction in extracellular levels of GABA after MPD administration, however this effect was not observed in EV rats. In addition, basal and stimulate glutamate levels in NAcc were not different between experimental groups. These results suggest that neonatal exposure to EV affect the neurochemical response to psychostimulants in adulthood, which could be a vulnerability factor to increase the doses of abuse drugs.

34. MITOCHONDRIAL EXPRESSION OF SVCT2 IN HIPPOCAMPAL NEURONS TREATED WITH OLIGOMERIC AB.

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Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive memory impairment and cognitive dysfunction. The most distinctive phenomenon correlating with AD is the presence of aggregates form of the β-amyloid (Ab) peptide. Experimental evidence has shown that the oligomeric form is able to induce reactive oxidative species (ROS). Ascorbic acid (AA) is an essential micronutrient with a preponderant role in oxidative stress and its main transport system in the brain is SVCT2, that incorporates the reduced form of vitamin C (ascorbic acid, AA), which is the prevalent form in vivo. Recent works show the relevance of AA in AD's progression by the inhibition of SVCT2, nevertheless, these studies didn't analyze how vitamin C is acquired by neurons and compartmentalized within organelles. In this work, we studied the subcellular localization of the SVCT2 transporter in primary cultures of mouse hippocampal neurons, and the changes in localization and expression levels associated to oligomeric Ab treatment. Material y methods: 18 days embryos hippocampus of C57BL/6 mice were dissociated and plated. Were cultured in vitro by 9 days until its treatment with oligomeric Ab. At 24 and 48 hours of treatment the subcellular localization of SVCT2 was evaluated, though immunofluorescence. Results: It was observed that hippocampal neurons show expression of SVCT2 which is located mainly at the mitochondria. Treatment with Ab oligomers increases the colocalization of SVCT2 with this organelle. Discussion: Our results suggest that mitochondrial AA might be relevant for neuronal survival in response to ab damage and suggest that this transporter would be a new therapeutic target to treat this disease.

35. NOVEL CAFFEIC ACID DERIVATIVES AGAINST ORAL CANCER CELLS.

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The cancer is a cellular process characteristic by uncontrolled growth and dissemination, being the second cause of death worldwide. Likewise, the oral cancer is one of the ten most common neoplasm in the world, mainly concern the tongue. The gold standard therapies are surgery, which can be complemented with radiotherapy and/or chemotherapy. The latter, represent the first-line therapies, involving decreasing tumor size and abolishing microscopic disease. However, this cancer has small average of survival, several side effects and drug resistances.

Therefore, new drugs are currently being studied, such as novel caffeic acid derivatives reporter before with antitumoral effect in breast cancer. We evaluated these new compounds by measurements of cytotoxic effect by MTT assay, mitochondrial effects by evaluation of mitochondrial-transmembrane potential by flow cytometry and ATP levels through luminescence assay. We showed that the derivates have cytotoxic effect in human cancer cell lines Cal27 and Hep-2. We found that the compounds generated the decrease in cellular ATP levels and decrease the measuring ATP levels on human cancer cell line Cal27 and Hep of the mitochondrial-transmembrane potential. In addition, we evaluated the selectivity of these compounds by exposing normal epithelium cells to their action by evaluation of cytotoxicity. Our results demonstrated the novel caffeic acid derivates exert a selective cytotoxic effect by mitochondrial mechanism.

36. CARDIOMYOCYTES HYPERTROPHY INDUCED BY CHRONIC STIMULATION WITH FRUCTOSE: BENEFICIAL EFFECT OF METFORMIN.

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Fructose intake has been increased in recent decades. Studies have indicated that high fructose consumption would be associated with an increase in the incidence of cardiovascular diseases (CVD) such as cardiac hypertrophy. Cardiac hypertrophy is an adaptive response to chronic work overload imposed on the heart, where cardiomyocytes undergo numerous morphological and functional changes. According to literature, fructose is able to decrease cell redox defenses and reduce adenosine monophosphate-activated protein kinase (AMPK) activation. AMPK activation is related to cardioprotective effects, and during hypertrophy allows the heart to change its metabolism. The objective of this work was to study the effect of fructose on cardiomyocyte hypertrophy and AMPK activation in the presence of metformin, a cardioprotective drug widely used in the treatment of type 2 diabetes mellitus. Cultured neonatal rat cardiomyocytes (1-3 days) were treated with 25 mM fructose at different times. The mRNA levels of hypertrophic markers (Beta-MHC, ANP, BNP and RCAN1.4) were determined by qRT-PCR. Phosphorylated -AMPK, total-AMPK and Beta-MHC protein levels were determined by inmunowestern blot (WB). The results showed that fructose increased mRNA levels of the hypertrophic markers and Beta-MHC protein levels. Further, it was observed that metformin was able to increase AMPK activation even in the presence of fructose which could prevent the progression of cardiomyocyte hypertrophy induced by this carbohydrate.

37. INDUCTION OF CELLULAR SENESCENCE IN PRIMARY ADULT MOUSE CARDIAC FIBROBLASTS.

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Introduction: Cellular senescence – a hallmark of aging – is related with the biomarkers appearance and up-regulation, such as senescence-associated beta-galactosidase activity (SABG), activation of tumor suppressor proteins (p53, p21CIP1) and increase of DNA damage associated proteins (gammaH2A.X). Recent studies suggested that interleukin-1beta (IL-1b) and doxorubicin (Dox) promote cardiac aging through an inflammatory process. Cardiac fibroblast (CF) keeps the extracellular matrix homeostasis and actively participates on damage-associated inflammatory and scaring processes that are altered in cardiac aging. Few studies have evaluated the increase of these biomarkers on CF by pro-inflammatory molecules such as IL-1b and Dox as potential inducers of cardiovascular damage. Objective: The objective is to evaluate IL-1b and Dox effects upon SABG, levels of tumor suppressor- and DNA damage-associated proteins as biomarkers of cellular senescence. Methods: CF were isolated from 8-10-week-old C57BL/6 male mice. CF were serum-starved by 24 hours prior to stimulation with IL-1b (2,5 ng/mL) and Dox (10 nM) for 24 hours. SABG was measured by microscopy with a commercial kit (Cell Signaling TECHNOLOGY®) and protein levels of p53, p21CIP1, and gammaH2A.X by immune-blot. Results: IL-1b and Dox increase the

percentage of SABG positive cells as long as the protein levels of p53, p21CIP1, and gammaH2A.X. Conclusions: IL-1b and Dox show an inductor effect of cellular senescence – featured by increased SABG and p53, p21CIP1 and gammaH2A.X levels – on CF.

38. NEUROPHYSIOLOGIC MODULATION OF NUCLEUS ACCUMBENS BY THE ACTIVATION OF SEROTONIN RECEPTOR 5-HT$_{2A}$

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Serotonin is a neurotransmitter implicated in most processes related to mood, sleep regulation, sexual behavior and cognitive modulation, being important in synaptic transmission as well. While, serotonin transmission is not completely, new evidences showed an important role for serotonin receptor activation in prefrontal cortex (PFC), dorsal raphe nucleus (DRN) and ventral tegmental area (VTA), specifically the activation of serotonin receptor 5HT2A. Electrophysiological studies in PFC showed that serotonin has effects on excitability profiles of pyramidal cells of layer V through the activation of 5HT2AR where its expression pattern is very high. These cells project to nucleus accumbens (Nacc; another nucleus with high expression of 5HT2AR), where the specific role of 5HT2AR activation is not known regarding synaptic transmission. To address this issue, we used electrophysiological whole cell patch clamp and current clamp techniques to record in acute coronal slices from Nacc of adult Sprague Dawley rats, MSNs in presence of serotonin and TCB-2 (agonist of 5-HT2AR). The experiments were made in presence of picrotoxin and tethrodotoxin to determine and characterize the specific activation of 5-HT2AR in MSN only of the excitatory inputs. Our results show a decrease in the amplitude of mEPSCs (miniature excitatory postsynaptic currents), which is related with a postsynaptic event, and no significant changes in the frequencies of mEPSCs. Also, we observed changes in firing rate and action potential threshold parameters in the presence of TCB-2, augmenting the firing rate and diminishing the action potential threshold. Taken together, the activation of this receptor within MSNs of the Nacc has effects on excitability parameters of these cells and also a possible modulation of serotonin and dopamine transmission within this nucleus.

39. MESENCHYMAL STEM CELL SECRETOME (MSCSS) ADMINISTRATION REDUCES OXIDATIVE STRESS AND NEUROINFLAMMATION INDUCED BY PERINATAL ASPHYXIA IN RAT HIPPOCAMPUS.

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Perinatal asphyxia (PA) is an important obstetric risk occurring at the time of delivery. Surviving children develop long-lasting motor and cognitive deficits. PA implies a primary energy crisis resulting from oxygen interruption, followed by a secondary insult linked to the required re-oxygenation, leading to oxidative stress, neuroinflammation and cell death, affecting basal ganglia and hippocampus. Neuroinflammation activates NF-kappa b signalling, which implies p65 nuclear translocation and pro-inflammatory gene expression, resulting in glial activation and apoptosis. Oxidative stress activates Nrf2, the antioxidant defense master regulator, promoting antioxidant gene transcription, including hemoxigenase 1 (HO-1) and NAD(P)H dehydrogenase quinone 1 (NQO1). MSCs have been proposed as potent agents to treat several conditions associated with neuroinflammation and oxidative stress. Preconditioning of MSCs with pro-inflammatory cytokines or hypoxic conditions improve their effectiveness. The aim of this study was to determine whether a single intranasal administration of secretome derived from preconditioned human MSCs to asphyxia-exposed rats activate (i) the Nrf2 pathway, (ii) reducing oxidative stress, (iii) neuroinflammation and (iv) cell death in rat hippocampus. Two hours after birth MSCSs (6 ug/16 ul, obtained from 1x10-6 preconditioned-MSCs) or 16 ul of vehicle were administered intranasally to asphyxia-exposed or control rats. Animals were euthanized at day P7. Oxidative stress was monitored by the GSSG/

GSH ratio, Nrf-2 nuclear translocation and HO-1 and NQO1 protein levels by Western blots (WB) and immunofluorescence (IFI). Neuroinflammation by microglial reactivity (anti-Iba-1, IFI) and p65 nuclear translocation (WB). Cell death by cleaved caspase-3 protein level (WB). The administration of MSCSs: (i) lowered the PA increased GSSG/GSH ratio, and (ii) increased both Nrf2 nuclear translocation and HO-1, NQO1 levels, (iii) reduced the microglial reactivity and nuclear P65 and (iv) reduced cleaved caspase levels.

40. A COMPUTATIONALLY GUIDED ANTIBODY AFFINITY OPTIMIZATION METHOD BASED ON MACHINE LEARNING SINGLE-POINT MUTANTS SELECTION.

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Antibodies are the biomolecules with therapeutic applications (TAbs) with highest growth and great effectivity. Currently, we know 78 monoclonal antibodies approved by the FDA, and many others are in last clinical stages. The applicability of antibodies depends on two properties: specificity, and affinity. Antibody affinity maturation is a natural process and can be experimentally emulated from diversity libraries and epitope selection, process called optimization. This selection is affected by external factors and unexplored conformational states, the discovered sequences have variable affinities with values we can improve. On the other hand, the next generation sequencing, the molecular three-dimensional modelling, and molecular docking have enabled perform rational antibody design without protein crystal complex. This process is assisted by computers and complex algorithm based on biophysical properties of the intermolecular forces that drive the binding energy, the interface between the epitope on the antigen, and the complementary determinants region on the antibody are dependent of the residues type and position in this no continuous loop. To improve and optimize the affinity we need explored the residues substitution and evaluate the energy free changes. The combinatory is about 10^9 unique sequences, while the free energy methods are computational requirement extensive. We develop a Abpred, an algorithm based in protein interfaces features and machine learning selection to propose a point-mutations combinatory to optimize the affinity. We train Abpred using an artificially-balanced dataset derived from SKEMPI-2.0; 1392 single-point mutations on 50 Ab-antigen complexes. Evaluation on blind-test (20% of dataset) achieved an RMSE of 1.66 kcal/mol, and 0.593 correlation. Abpred enable the affinity optimization in silico to accelerate the design of news TAbs.

41. SELECTION OF MOLECULES FOR THE FUNCTIONALIZATION OF GENE-CHARGED-GOLD-NANOPARTICLES THAT ACHIEVE TARGETING OF PROSTATE CANCER CELLS.

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Metal nanoparticles (NPms) have attracted the interest of biomedical researchers due to their potential application in the diagnostic and treatment of diseases like cancer. These NPs can enter the cell depending on their size, charge, and surface functionalization. NPs between 10 to 100 nm have a large surface area that can be functionalized with different molecules for targeting (antibodies, ligands of cell surface proteins), detection (fluorescent probes) and treatment (drugs, proteins, nucleic acids) of affected cells. In this way, NPs could allow detection of target cells and be a therapy, at the same time. Such NPs has been described as ¨theranostic NPs¨. Among molecules used to successfully target cancer cells are folic acid, RGD peptides, and EGF-receptor antibodies. In this work, we show the formulation and characterization of gold nanoparticles designed for gene delivery treatment of prostate cancer cells. Selectivity for prostate cancer cells of NPms functionalized with folic acid or anti-LOX-1 antibody were compared. These NPms were tested in four prostate cancer cell lines: LnCap, Du-145, C4-2B, and PC3 while RWPE-1 was used as a control cell line. Molecules that mediate cell targeting of NPs are determinant in the success of theranostic nanoparticles. These NPs could favor cancer early detection, improving treatment response and/or achieving a complete recovery.

42. INFLUENCE OF DIVERSE FACTORS ON ADHERENCE TO ORAL ANTIDIABETIC MEDICATION IN MEXICAN PATIENTS WITH TYPE 2 DIABETES.

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Several factors can affect adherence to pharmacological treatment in patients with type 2 diabetes (T2D), including gender, the time of evolution of the disease, and the beliefs of the patient about health. The aim of the present study was to examine the main factors that influence adherence in T2D patients affiliated with a public hospital in Chiapas, Mexico. A cross-sectional study was carried out on a sample of 200 patients being treated with oral hypoglycemic agents, of which 50% were women and the average age of the women was 61.2 ± 7.7 years and of the men 65.2 ± 1.4 years. The patients were asked to provide the most recent result of fasting glycemia and to fill out a sociodemographic questionnaire, the Morisky-Green test and the Beliefs about Medicines Questionnaire (BMQ). The possible associations among the variables were analyzed by the Chi-squared test, while the comparison of the results of the BMQ between compliant and non-compliant patients was examined with the Mann-Whitney U test. The results show that 77% of the women and 85% of the men presented a lack of adherence to medication. The habit of smoking had a significant association with the lack of adherence. The men and women who were compliant mentioned a greater need for the medication ($U1=369.50$, $U2=553$; $p<0.005$). However, only the compliant women had levels of glycemia significantly lower than the group of non-compliant patients ($t=757$; $p<0.05$). Hence, it is possible that the responses of the men were more prone to falsehoods than those of the women. This could justify a future revision of the instruments used to test the adherence of men.

43. CYTOTOXICITY OF NITROFURANS AND C-5 SUBSTITUTED FURANS. EVIDENCE OF NITROREDUCTION-INDEPENDENT CYTOTOXIC EFFECTS.

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Nitrofurans constitute a family of drugs that have antibacterial and antiparasitic effects. The consumption of these causes various adverse effects which are mainly attributed to the monoelectronic reduction of the 5-nitro group mediated by host enzymes. This triggers the generation of nitroanion radical which, upon entering redox cycling with molecular oxygen, results in the formation of superoxide anion radical and the regeneration of the nitro derivative. The generated oxidative stress allows to explain, at least in part, the adverse effects attributed to nitrofurans. However, not all side effects are attributable to that mechanism. In order to demonstrate that the cytotoxic effects of nitrofurans are not solely due to the reduction of the 5-nitro group, derivatives of 3 commercial nitrofurans (nitrofurazone, nitrofurantoin and nifuroxazide) were synthesized in which the 5-nitro group was substituted by Methyl, Bromo or Hydrogen, maintaining the parental skeleton. The synthesis was carried out in aqueous solution through ultrasound, mixing the aldehydes with the corresponding hydrazines. Subsequently, the effect of these derivatives on cell viability and their ability to generate intracellular ROS was evaluated. The results show that the cytotoxic effect of furan derivatives is dependent on the presence of electron withdrawing groups in position 5 of the furan ring; while the generation of intracellular ROS does not depend exclusively on the presence of the 5-nitro group. This suggests new toxicity mechanisms independent of oxidative stress induced by redox cycling of nitrofurans.

44. ELEPHANT BLACK GARLIC EXTRACT PREVENTS MITOCHONDRIAL DYSFUNCTION INDUCED BY BETA-AMYLOID PEPTIDE IN MOUSE HIPPOCAMPAL SLICES.

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Soluble oligomers of the beta-amyloid peptide (SO-Aβ) are central elements in the pathogenesis of Alzheimer's Disease (AD). It has been demonstrated that SO-Aβ forms pores in the plasma membrane which leads to Ca2+ influx and leakage of large molecules, such as ATP. Cytosolic Ca2+ overload induces mitochondrial dysfunction and synaptic failure resulting in cell death. Some studies suggest that common black garlic (Allium sativum) prevents the toxicity of SO-Aβ due to its enriched composition of sulfur metabolites. However, there are no studies on the biological activity of elephant black garlic (Allium ampeloprasum), and recently in our laboratory we identified new sulfur compounds that are not present in common garlic. The objective of this work was to evaluate neuroprotective properties of elephant black garlic extract (BG) against SO-Aβ toxicity. In mouse hippocampal slices, BG (20 µg/mL) prevented the decrease in cellular viability induced by SO-Aβ (2.5 µM) by 60±6%. In parallel, BG kept the mitochondrial membrane potential stable compared with SO-Aβ (SO-Aβ: 67±3%; SO-Aβ+BG: 96±5%), suggesting a direct effects on mitochondrial function; additionally, the intracellular (SO-Aβ: 70±6%; SO-Aβ+BG: 110±9%) and extracellular ATP levels (SO-Aβ: 180±23%; SO-Aβ+BG: 125±16%), were recovery when BG was present. We also observed that BG normalized the levels of the mitochondrial fusion protein MFN1 (SO-Aβ: 49± 10%; SO-Aβ+BG: 91±13%). On the other hand, our results showed that BG preserved the synaptic structure and prevented the decrease in SV2 (SO-Aβ: 64±4%; BG: 97±9%) and PSD95 proteins (SO-Aβ: 45±8%; SO-Aβ+BG: 76±7%) and also the decrease on transient intracellular calcium induced by SO-Aβ (54±5%). Finally, our results suggest that the bioactive compounds present in BG could be new pharmacological tools to treat the SO-Aβ toxicity.

45. ROLE OF ROCK-1 AND ROCK-2 IN ADHESION AND MIGRATION OF TRYPANOSOMA CRUZI- ACTIVATED MACROPHAGES TREATED WITH ATORVASTATIN.

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Chagas Disease (CD) is caused by protozoan Trypanosoma cruzi (T. cruzi). The most severe CD clinical manifestation is Chronic Chagas cardiomyopathy (CCC). Currently treatment is benznidazole, a trypanocidal therapy, ineffective against CCC. Therefore, is interesting to develop new therapeutic pharmacologic strategy. It has been observed in autopsies of patients with CCC that present an important leukocytes infiltration in cardiac tissue, and more of 50% are macrophages. The macrophage infiltration needs the macrophages adhesion and migration from the vascular endothelium to damage site. Rho-associated protein kinase (ROCK) 1 and 2 are serine-threonine kinases activated by small GTPase RhoA. ROCK phosphorylate myosin light chain (MLC), promoting cellular contraction and generating focal adhesion. On the other hand, ROCK phosphorylate cofilin, increasing actin polymerization. Therefore, ROCK activation affects cellular migration and adhesion. In our laboratory we have observed, T. cruzi activates ROCK in human macrophages, leading to proinflammatory phenotype and atorvastatin inhibited ROCK, changing the phenotype of this cells. However, it has not been studied ROCK-1 and ROCK-2 role in T. cruzi effect and atorvastin over macrophages adhesion and migration. Human macrophages U937 were used, with a knockdown for ROCK-1 and ROCK-2 and with both proteins constitutively active by nucleofection. T. cruzi-activated macrophages were treated with atorvastin and adhesion process to endothelial cells (HUVEC) were determined by microscopy and western blot. Also, migration was studied by microscopy time lapse.

It was observed that both isoforms are involved in adhesion and migration increase promoted by T. cruzi and is inhibit by atorvastin. These results allow us to propose atorvastin as a therapy to decrease macrophages infiltration in CCC.

46. IMPAIRMENT OF SPIKE TIMING-DEPENDENT PLASTICITY IN PREFRONTAL CORTEX IN A KETAMINE TREATED MICE.

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The medial prefrontal cortex (mPFC) is a key structure involved in cognitive functions like working memory and decision-making. Long-term changes in synaptic plasticity, i.e: long-term potentiation (LTP) and long-term depression (LTD) have been proposed as the cellular substrate of these cognitive processes. During the postnatal development, the maturation of mPFC circuits depends largely on the network of inhibitory interneurons, which modulate the pyramidal neurons (PYN) function. The mPFC interneurons are especially vulnerable to injury during adolescence and the impairment of GABAergic interneurons function is involved in several neuropsychiatric diseases, including schizophrenia (SZ). However, it is still unknown how the disruption of GABAergic interneurons development during adolescence can affect the long-term synaptic plasticity in the adult brain. Using electrophysiological and pharmacological approaches, we evaluate the efficacy of synaptic transmission and spike-timing-dependent plasticity in mPFC in an SZ mice model based in the adolescence treated with non-competitive NMDAR antagonist ketamine (Ket). Through recording in PYN of the layer II / III of mPFC slices in adulthood, we found that the frequency of spontaneous and miniature inhibitory post-synaptic currents (sIPSC and mIPSC) was lower in Ket treated animals than control. Also, we observe that the paired-pulse ratio of eIPSC was higher in Ket mPFC slice than control. Using the STDP protocol we found that while the protocol in t-LTP induced a similar potentiation that in control, the t-LTD protocol was unable to induce depression, conversely it can induce LTP. These data suggest that hypofunction of NMDAR that impair the GABAergic interneurons maturation and function, decrease the GABA release, which can reverse the temporal dependence of STDP-LTD modifying the synaptic plasticity and function of mPFC network in adulthood mice.

47. CHARACTERIZATION OF THE ADIPOGENIC PROTEIN E4ORF1 FROM ADENOVIRUS 36 THROUGH AN IN-SILICO APPROACH.

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Adenovirus 36 (Ad-36) is related to human obesity due to its adipogenic activity mediated by the Early 4 Open Reading Frame 1 (E4orf1) protein. Mechanisms underlying adipogenic effect of E4orf1 are not completely understood; however, it has been characterized the increased proliferation and differentiation of fat cells through the activation of the Phosphatidyl Inositol 3 Kinase pathway by binding proteins containing PDZ-domains. We aimed to characterize E4orf1 structure and analyze its interactions with PDZ-domain containing proteins in order to recognize important residues with pharmacological purpose. In-silico approaches such as homology modeling, molecular dynamics and molecular docking between E4orf1 and five PDZ-domains from different proteins (PDZ-1 and 2 from Disk Large Homolog 1; PDZ-3 from Membrane Associated Guanylate Kinase 1; and PDZ-7 and 10 from Multy PDZ-Domain Protein 1) were performed. Mutagenesis of selected residues was performed to evaluate its importance in the stabilization of E4orf1:PDZ complexes. We predicted the first 3D model of E4orf1, which suggests a key role of residues at c-termini region (114 to 125), demonstrating its importance in initial stabilization. The complex formed by predicted E4orf1 and PDZ10 was more stable than others. Moreover, residues at “core” region (residues 80 to 85) in E4orf1:PDZ10 complex were important in stabilization as demonstrated by its electrostatic interactions. Mutagenesis highlighted residues 80-85, demonstrating its importance in complex stabilization. In conclusion, E4orf1 forms a stable complex with PDZ10 domain, being residues 80-85 of particular importance. Characterization of E4orf1 interactions provides a first approach in discovering druggable targets for Ad-36 induced obesity.

48. INTERFERON AS THERAPEUTIC CANDIDATE FOR CANINE VIRAL DISEASES.

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Chilean dog population is estimated in 3,2 million, with a continue growing in pet market associated with care products. In health expenditure, vaccines reach 25% of total cost in pet's care. Among pathologies that affects pets, viruses are the main source of infectious diseases, including distemper, parvovirus and tracheobronchitis. Vaccines against parvovirus are currently available, however still is considered one of the most important disease worldwide, with a high prevalence level in our country. Nowadays, there is not a large spectrum and specifics antiviral drugs, having to use human antivirals or from another species. Specifically, cytokines used as therapeutics activates immune system and helps to defense against virus, preventing replication and infection. In this work we developed a prototype drug for canine specifically antiviral treatment, based in a recombinant dog-derived interferon. The molecule was produced in a E. coli expression system. Besides, specific interferon activity was measured by OAS-2 and PKR mRNA quantification, in Madin Darby Canine Kidney (MDCK) cell line and dog lymphocytes primary cultures stimulated with Canine recombinant interferon was obtained with purity around 88% and the antiviral markers were enhanced in canine cells, hence the cytokine could be an option for antiviral therapy in canine population.

49. THE GLUCOSE TRANSPORTER (SGLT-2) AS A POSSIBLE URINARY MARKER OF EARLY DIABETIC NEPHROPATHY.

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Hyperglycemia is characterized by high blood glucose concentrations (≥126 mg / dL) and over time these glucose levels damage renal microvasculature. Diabetic nephropathy is the final complication of this pathology, where well-characterized morphological and functional alterations occur. At present there is no specific and early urinary marker that allows determining the risk of developing diabetic nephropathy once diabetes is established. Therefore, the objective of this work was to evaluate SGLT-2 as a specific urinary marker of renal damage in a murine model with early diabetic nephropathy. Type 1 diabetes was induced in Wistar rats by administration of streptozotocin (60 mg / kg, ip). Diabetic rats were sacrificed 21 days after induction. Proteinuria and creatinine were determined as renal function tests. Western blotting analyzed the expression of SGLT-2 and β2-microglobulin. An increase in the urinary excretion of SGLT-2 was found in the third week of damage with diabetic nephropathy and the presence of β2-microglobulin in the urine as a marker confirmed the presence of damage. These results suggest SGLT-2 as a new urinary marker of diabetic nephropathy that allows predicting the risk of developing diabetic nephropathy. Keywords: Diabetic nephropathy, streptozotocin, β2-microglobulin, SGLT-2

50. TMA-6(2,4,6-TRIMETHOXYAMPHETAMINE) MODULATES NEUROPLASTICITY IN THE PREFRONTAL CORTEX AND ENHANCES HEAD-SHAKES BEHAVIOR IN SPRAGUE-DAWLEY RATS.

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The search for new analogs of the entactogen MDMA (3,4-methylenedioxy-methamphetamine, "ecstasy") is of great relevance for the development of new drugs useful in the treatment of prevalent neuropsychiatric diseases. While the central effects described for MDMA clearly differ from other psychotropic

compounds, it has been proposed that the presumed low potency hallucinogen TMA-6 (2,4,6-trimethoxyamphetamine) might induce MDMA-like effects at low doses in humans. Since both hallucinogens and MDMA might exert different effects on cognitive processes, one may anticipate differential effects with respect to neuroplasticity at key sites of the central nervous system. Here we compared the acute effects of TMA-6 with MDMA on (i) in vivo induction of long-term potentiation (LTP) in the prefrontal cortex of the rat, and (ii) induction of paroxysmal head rotations termed "head-shakes", which is considered a behavioral proxy in rodents for human hallucinogenic-like effects. The results obtained showed that 20 mg/kg TMA-6 not only prevented the induction of LTP in the prefrontal cortex but turns it into a long-term depression-like event. In addition, TMA-6 significantly increased the number of head-shakes, verifying the hallucinogenic nature of this compound. In contrast, 10 mg/kg MDMA significantly increased the prefrontal cortical LTP but fully abolished the number of head shakes. Taken together, and unlike the presumption based on the subjective interpretation of its effects in humans, the inverse electrophysiological/behavioral profile of TMA-6 referred to MDMA suggests that the former seems to lack entactogenic-like effects, rather supporting the hallucinogenic essence of this drug.

51. SITE-SPECIFIC PEGYLATION OF L-ASPARAGINASE: AN ALTERNATIVE FOR THE TREATMENT OF ACUTE LYMPHOBLASTIC LEUKEMIA.

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L-asparaginase (ASNase) is a therapeutic enzyme considered a cornerstone in the treatment of Acute Lymphoblastic Leukemia (ALL), the most common cancer in children worldwide. Four formulations of bacterial origin are available in the market for the treatment of ALL. However, despite their effectiveness, they generate immunological reactions, decreasing the action of the drug and damaging patient safety. PEGylation is one of the strategies adopted to reduce the immunogenicity of asparaginase, which consists of the covalent binding of polyethylene glycol (PEG) chains to the enzyme, at specific or random sites. This allows reducing the recognition of the enzyme by the immune system and its early elimination from the bloodstream. In this work, we performed the N-terminal PEGylation of E.coli ASNase, with methoxy polyethylene glycol-carboxymethyl N hydroxysuccinimidyl ester (mPEG-NHS 10kDa) in 100 mM PBS at pH 7.5 and PEG: ASNase ratio of 25:1. As a result, we obtained the monoPEGylated ASNase with an activity of 134 ± 11.5 IU/mg and acceptable kinetic parameters. MonoPEGylated ASNase also showed more stability at different pH, temperatures and against human serum proteases than the native enzyme, demonstrating its potential as a less immunogenic biopharmaceutical in the treatment of ALL.

52. CHARACTERIZATION OF CA125 ANTIGEN EXPRESSED IN HUMAN CERVICAL CARCINOMA CELLS.

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Epithelial ovarian cancer is the seventh death cause related to cancer in women worldwide. Due to the absence of early stage clinical symptoms, patients are usually diagnosed when decease had spread further than ovary with an unfavorable prognostic. Cancer antigen 125 (CA125) is a serum marker extensively used in gynecology for monitoring epithelial ovary cancer patients. It's a repetitive peptide antigen of the membrane glycoprotein MUC16, whose over-expression in ovarian cancer has been linked with both pro-metastatic and pro-tumorigenic properties. In the present study, we characterized the N-oligosaccharides bonded to the C-terminal region of MUC16, expressed in cervical carcinoma cell culture by adenoviral transduction. Enzymatic deglycosylation, HPLC, and MADI-TOF analysis showed mainly complex type of oligosaccharide N-linked, with bi-antennary, mono-sialylated and mono-focusylated core structures, predominantly. It has been previously reported that N-glycan profiles from cancer patients shows an increase of these structures, compared to healthy groups. Furthermore, these N-glycan structures have been described as part of innate and adaptive immune response recognition by CA125 antigen. Concluding, the glycosylation status

of the CA125 may provide specific biomarkers and therapeutic target for gynecologic cancer.

53. DESIGN, SYNTHESIS AND BINDING AFFINITIES OF CYCLOALKYLAMINES AND PIPERIDINES ESTERS DERIVATIVES ON ALPHA4BETA2 NICOTINIC RECEPTOR AND MONOAMINE TRANSPORTERS (HSERT AND HDAT).

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The monoaminergic and cholinergic neurotransmitter systems exhibit, in the central nervous system (CNS), a wide range of functional interactions and mutual regulations. Furthermore, acetylcholine (ACh) actions mediated by nicotinic receptors (nAChRs), as well as monoamines such as serotonin (5-HT) and dopamine (DA), are involved in the modulation of several brain functions, including (but not limited to) cognition, voluntary movement, motivation and reward, mood, attention and learning, as well as in the physiopathology of a variety of diseases. In addition, most of the drugs currently used for the treatment of neurological and neuropsychiatric disorders such as Parkinson`s and Alzheimer`s diseases, depression, drug addiction, schizophrenia, etc., have mechanisms of action associated to the regulation of one or more of these systems. Indeed, there are some examples of therapeutically useful drugs, which act through simultaneous interactions with SERT/DAT and nAChRs. Therefore, it seems attractive to search/formulate ligands that show such a promiscuous profile fusing structural aspect of nicotinic ligands and antidepressants. Here, we design and synthesize cycloalkylamines and piperidines esters derivatives. Binding experiments were assessed for alpha4beta2nAChR on brain synaptosomes and hSERT and hDAT from specific cell lines. The ester moieties were acetyl, propionyl and benzoyl derivatives in order to study steric effect into the binding site. Our results indicate that, some compounds are able to displace radioligands from nicotinic receptor and MAT, showing a promiscuous behavior. However, the ranges of affinities were in the micromolar order. In addition, docking experiments were performing in order to rationalize the binding mode and the similar interaction between nAChR, SERT and DAT with our compounds.

54. RATIONAL DESIGN AND BIOLOGICAL EVALUATION OF TRIAZOLOPYRIDINES AGAINST T. CRUZI.

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Since a while ago, Azole-containing compounds have been recognized for their antifungal features and with well-known applications in current clinical field. Some of features highlighting azole heterocycle come from its specific metabolism, those related to its high binding affinity to heme-containg proteins such as cytochrome p450. The p450 complex has the function of metabolizing drugs, exogenous and endogenous molecules that could render in cell toxicity. Lanosterol 14α demethilase (CYP51) is the key enzyme in sterol biosynthesis in Trypanosoma cruzi (Tc), agent that causes Chagas disease. The inhibition of this enzyme will induce accumulation of toxic metabolites that cause the death of the parasite. Recently, we have synthesized and characterized derivatives of triazolo pyridines, based on a rational study (structure-activity), where we found that compound 1 has antiproliferative effect against the replicative form. From this structure, we obtained 24 molecules with variations in positions 3 and 7 in the ring [1,2,3] triazolo [1,5-a] pyridine with different electrophiles such as pyridines, thiophenes, benzenes and pyrazines. Studies (structure/activity) of this new series have shown that the compound 2 increased the trypanocidal potency. On the other hand, preliminary

docking studies showed that the association energy of the compound to CYP51 are similar to the binding energy values of fluconazole to CYP51, our approximations have shown that both the azole fraction and pyridine are potentially related to the coordination with hemo. This has led to design of a new proposal of antichagasic drugs with a possible action on CYP51.

55. EXPLORING NOVEL ALLOSTERIC MODULATORS OF ALPHA3 GLYCINE RECEPTORS: STRUCTURE-ACTIVITY RELATIONSHIP OF 2,6-DI-TERT-BUTYLPHENOL DERIVATES.

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Glycine Receptors (GlyRs) are pentameric anion-permeable ligand-gated ion channels highly expressed in spinal cord and brain stem, where mediate processes such as motor coordination and sensorial processing. The modulation of glycine receptors composed by alpha3 subunit (a3GlyRs) by positive allosteric modulators (PAMs) has been associated to generation of analgesia in chronic pain models. Previously, we shown that the non-sedative propofol analog 2,6-di-tert-butylphenol (2,6-DTBP) is a PAM of a3GlyRs. Here, we analyzed series of 2,6-DTBP analogs using bioinformatics and electrophysiological methods. Whole-cell recordings shown that 2,6-DTBP (0,1 mM) potentiate a3GlyRs-evoked currents in a 171±21%. The first set of experiments evaluated the impact of the tert-butyl group positions around the phenolic core. These studies concluded that the presence of two tert-butyl groups at the position 2 and 4 around the phenolic core (i.e. 2,4-DTBP) were sufficient to generate an a3GlyR PAM with a significantly higher efficacy. A second set of molecules evaluated the impact of additional chemical groups on the 2,4-DTBP scaffold (i.e. methyl, ethyl, oxime, amine, carboxylic, sulfonamide groups). Whole-cell recordings shown that ≈30% of these compounds displayed improved efficacy in comparison to 2,6-DTBP (≈3-4 fold of a3GlyR potentiation). Bioinformatics analysis shown that all the compounds evaluated have an ADME profile compatible with drugs-like molecules. Pharmacophore modeling shown that the localization of polar, hydrophobic and charged groups within the 2,4-DTBP is pivotal for the a3GlyR modulation. Through a systematic analysis of 26 analogs of 2,6-DTBP, the present work allowed the identification of novel a3GlyRs PAMs with improved efficacy. In addition, the generation of a pharmacophore based on these findings expand the possibilities for further design and development of new GlyRs PAMs.

56. ATORVASTATIN AND ROSUVASTATIN TREATMENT CONTRIBUTES TO THE DECREASE OF PROLIFERATION IN HUMAN UMBILICAL ARTERY SMOOTH MUSCLE.

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The treatment of choice for hypercholesterolemia is the use of statins. Enzymes that inhibit the enzyme 3-hydroxy-methylglutaryl coenzyme A reductase, which is a limiting factor in the biosynthesis of liver cholesterol. Despite this, clinical studies have revealed that statins can exert atheroprotective effects, beyond the decrease of serum cholesterol. These effects have been called pleiotropic effects and include anti-proliferative and anti-migratory properties in vascular smooth muscle cells, which are a key cell type in atherosclerotic plaque development. For this reason, Human Umbilical Artery Smooth Muscle cultures were stimulated with 10 ng/ml of platelet-derived growth factor and treated with 20 µM atorvastatin and 20 µM rosuvastatin for 24 hours. The proliferative effect was subsequently evaluated for 48 hours by spectrophotometry using the CellTiter 96® AQueous One Solution Reagent colorimetric assay. The migratory effect was also evaluated for 4 hours by optical microscopy using transwell with 8 µm pores. Migrated cells were counted by means of ImageJ software with automated macros function commands. It was observed that 20 µM statins treatment reduces cell proliferation in a 48-hour period (p=0,05). However, there is no decrease in migratory cell capacity after being treated with atorvastatin (p=0,05) and rosuvastatin (p=0,05). For this reason, atorvastatin and rosuvastatin treatments might contribute to the proliferation reduction in smooth muscle cells, which are involved in atherosclerotic plaque formation. Financial support: FONDECYT 1171765 & DIUFRO DI19-2018

57. EXPRESSION AND CHARACTERIZATION OF A NOVEL SINGLE-CHAIN ANTIBODY AGAINST VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN GOAT MILK.

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Tumor angiogenesis is a hallmark of cancer and plays a significant role in establishing a vascular supply within the tumor which is essential for tumor growth and metastasis. The vascular endothelial growth factor (VEGF) plays an important role in angiogenesis process promoting endothelial cell proliferation, migration, and invasion. VEGF is overexpressed in many solid cancers including lung cancer, one of the most common cancers in the world. According to this, different approaches have been developed to suppress tumor angiogenesis, including anti-VEGF monoclonal antibodies as part of the cancer immunotherapy strategy. However, high production costs limit the widespread access to this treatment. In this study, we designed a novel single-chain monoclonal antibody (anti-VEGF) that can bind to VEGF. This antibody can be efficiently expressed in the mammary gland of goats by adenoviral transduction and purified from their milk. Results showed that anti-VEGF was able to avoid cells migration in a wound healing test and suppressed VEGF-induced microvessel sprouting in rat aortic ring assay. Furthermore, in vivo efficacy was evaluated on a xenograft lung tumor model where anti-VEGF treatment had an inhibitory effect on tumor growth. Our findings suggest the therapeutic potential of anti-VEGF as an anti-tumor agent correlated with suppression of angiogenesis.

58. DIVERSITY LIBRARY SEQUENTIAL SCREENING TO IDENTIFY COMMON DESCRIPTORS OF THE SGLTS INHIBITORS.

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Type II Diabetes Mellitus (T2DM) have an estimated 8.5% of the world adult population affected in 2014 and a projection of reaching 17.9% in 2060. Many different approaches have been developed for tackling the no-insullinic management of hyperglycemia in the diabetic patient, exploring the diverse variety of targets that the multifactorial nature of T2DM offers, which includes classicals insulin sensitizers like metformin, and also new pharmacological developments. One new approach to control T2DM is the use of sodium-glucose transporter 2 (SGLT2) inhibitors. While SGLT1 is involved in intestinal glucose intake, SGLT2 is located in the proximal tubules of kidneys, and is responsible for most of the glucose reabsorption (>90%). Inhibition of SGLT2 in combination with traditional treatments have shown improvements in the control of glycemia and a concomitant reduction in cardiovascular risk. Challenges in the development and extension of the chemical space associated with the inhibitory activity of SGLT2 are the high costs in time and money of the in vitro research and the lack of the human transporter atomic coordinates, due to the difficulties to crystallizing membrane proteins. These two problems can be addressed through a methodology that includes an exploration of molecular dynamics from the protein transporter and the structure-based virtual screening. We have developed a robust pipeline perform a virtual screening through massive molecular docking against a diversity library of molecules for the exploration of the chemical space for the discovery of new inhibitors. We use SGLT2 and known inhibitors to perform a sequential refinement to optimize the identification from a not supervised methods and extend the methodology to the other potential candidates transporter with therapeutics applications.

59. CHARACTERIZATION OF THE INTESTINAL MICROBIOTA OF PEOPLE TREATED WITH INFUSIONS OF ACAENA SPLENDENS AND ITS ASSOCIATION WITH THE INFLAMMATORY STATUS.

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Given the importance and usefulness of medicinal plants as therapeutic agents,

the Chilean Ministry of Health, under the guidance of the world health organization (WHO), has been working in recent years on the regulatory framework of the policy of Complementary Medicine and Traditional Herbal Medicines with clinical evidence support. Acaena splendens (Amores secos) has been used in Chilean traditional medicine for the treatment of fever and inflammation. In addition, an infusion of Acaena splendens, similar to that recommended by folk healers, exhibited highly significant antiinflammatory activity (46.7%) in a murine model of inflammation. Additionally, there is an emerging body of work on the human gut microbiome and how it mediates feedback between the foods we eat and our bodies. The gut microbiome is also an important mediator of inflammation in the gut and systemically. Considering that the mechanism(s) by which Acaena splendens modulate systemic inflammation are unknown, we propose that infusions of Acaena splendens could decrease systemic inflammation through changes in human gut microbiome. To test this idea thirty healthy volunteers will consume an infusion prepared from Acaena splendens for six and twelve days and blood/fecal samples will be obtained before and after the treatment for microbiome analysis (Illumina 16S rRNA sequencing and Oxford Nanopore MinIon) and inflammatory markers analysis (cytokine array kits and ELISA cytokine kits). The results from this proposed study related with the effect of medicinal plants on the composition of the intestinal microbiota and its association with systemic inflammation could provide new therapeutic strategies for treatment of inflammation-related diseases (e.g. colorectal cancer) a condition of high prevalence in the Atacama region.

60. STUDY OF A MOLECULE THAT INTERFERES IN GBΓ BINDING WITH THE CYTOPLASMIC DOMAIN OF GLYCINE RECEPTOR α1.

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Ethanol is the most widely used drug of abuse in the world. Its effects go from desinhibition, headaches, nausea, vomiting, even respiratory depression and death. Recently, the glycine receptor (GlyR) has been identified as one of the targets in which this drug acts, enhancing its inhibitory activity. This mechanism involves the interaction of the cytoplasmic domain of GlyR (GlyR-DC) with the βγ dimer of the G protein (Gβγ). Through bioinformatic studies, molecule M554 was selected, which binds to Gβγ at the same site of interaction for GlyR-DC inhibiting the effects of ethanol in vitro and in vivo. In this project it was studied whether this molecule inhibited the interaction between these 2 proteins. For this objetive, a fusion protein of GlyR-DC and Glutathione S-transferase (GlyR-DC-GST) was expressed and purified. Comparative studies of GST pull-down showed that GlyR-DC-GST retained its ability to interact with Gβγ. At the same time GlyR-DC was incubated with cell extracts, and the affinity of GlyR-DC with Gβγ in the absence and in the presence of 200 μM M554 was compared. Densitometric analysis allowed to determine that the interaction between both proteins effectively decreased in the presence of this molecule. Therefore, these results show that this molecule decreases the binding capacity of Gβγ with GlyR-DC, leaving clear that this is the basal mechanism for the inhibition of ethanol effects and supporting the projections that M554 could have a pharmacological potential to treat acute ethanol intoxication.

61. COMPLEX INHIBITION OF OXPHOS AND A-KETOGLUTARATE DEHYDROGENASE COMPLEX BY GENTISIC ACID-TPP+ INDUCES CELL DEATH IN BREAST AND LUNG CANCER CELL LINES.

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Cancer cells have a more hyperpolarized mitochondrial membrane potential than normal cells, which allows selectively guide towards mitochondria small cationic molecules such as triphenylphosphonium (TPP+), which participate as chemical chaperones for pharmacophores moieties. This property was used to synthesize from the natural product, gentisic acid, derivatives bound to TPP+ (GA-TPP+C10). This mitocondriotropic compound is capable of produce a time-dependent complex inhibition of mitochondrial bioenergetics characterized by 1) initial phase of mitochondrial uptake with uncoupling effect of oxidative phosphorylation, 2) inhibition of complex I-dependent respiration and, 3) a late phase of mitochondrial accumulation with inhibition of alfa-ketoglutarate dehydrogenase

complex activity. This complex is part of the tricarboxylic acid cycle composed of three subunits that oxidizes and decarboxylates alfa-ketoglutarate which is necessary for the synthesis of aspartate, an essential amino acid to proliferation and cell survival. The above was verified using human breast and lung cancer cell lines, by the addition of exogenous permeable metabolites: alfa-ketoglutarate (dm-alfaKG), aspartate (m-Asp) and pyruvate (pyr). It was shown that dm-alfaKG and m-Asp, but not pyr, produce a decrease in cell death caused by GA-TPP+C10. Moreover, the bioenergetic crisis induced triggers a drastic mitochondrial membrane potential drop, G1-phase cell cycle arrest with a significant increase in ROS. In addition, this blockade of mitochondrial functions triggers a metabolic remodeling toward glycolysis and pro-survival AMPK activation. Our results describe an anti-cancer mechanism of GA-TPP+C10 that induce a complex inhibition of mitochondrial bioenergetics in a time-dependent manner in breast and lung cancer cells that may have relevance at therapeutic level.

62. ELECTROPHYSIOLOGICAL RECORDINGS OF 3-(PYRIDIN-3-YLMETHOXY)QUINUCLIDINE (Q-01), A NOVEL SELECTIVE COMPOUND FOR THE A7B2 NICOTINIC ACETYLCHOLINE RECEPTOR.

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A novel heteromeric alpha7beta2 nicotinic acetylcholine receptor (nAChR) with functional properties different from those of alpha7 and alpha4beta2 nAChRs, was recently identified. Although its functions are not known, it appears this nAChR may be involved with Alzheimer's disease. To date there are no synthesis reports of ligands for alpha7beta2 nAChR. Our work was based on the design and synthesis of new ligands, as well as on establishing their possible effects with electrophysiological records in interneurons from the stratum radiatum of the rat hippocampal CA1 region. Thus, of eleven synthesized ligands, only Q-01 and EQ-01 inhibited the Choline-induced ionic current: 51 and 100%, respectively; that is to say, these acted as antagonists of alpha7 and alpha7beta2. However, Q-01 presented an inhibition similar to dihydro-beta-eritroidine (selective antagonist of nAChR containing the subunit beta2); suggesting that Q-01 might be more selective for alpha7beta2 nAChR than EQ-01. To understand the electrophysiological results, molecular docking studies were performed for the compound Q-01 at the nAChRs α7 and alph7beta2. These studies suggest that Q-01 would be more selective by subtype alpha7beta2.

63. STRUCTURE-BASED VIRTUAL SCREENING STUDIES TO IDENTIFY NOVEL POTENTIAL AGONISTS FOR SALMO SALAR GHSR1A-LR.

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Ghrelin is a growth hormone (GH) secretagogue and functions primarily as a GH-releasing hormone and as an orexigen. It has also been documented to be involved in the immune system, stress response, energy metabolism and growth in fish. Its receptor, Growth hormone secretagogue receptor (GHS-R), is a class A G protein-coupled receptor (GPCR) mostly expressed in the hypothalamus. In Atlantic salmon (Salmo salar) and other salmonids, a ghrelin receptor isoform called GHSR1a-LR has been identified. This receptor has an unique characteristic: the second extracellular loop (ECL2) is notably longer than in others GHS-R. In particular, GHSR1a-LRs have the characteristic that ghrelin or GH secretagogues treatment either does not increase intracellular Ca2+ or requires pharmacological doses to activate the receptor. Given the high conservation in folding and topology of class A GPCR receptors, a comparative model of GHSR1a-LR receptor from Salmo salar was generated. This model was based on the crystallographic structure of Neurotensin-1 receptor in active-like conformation (PDB ID: 4XES). Subsequently, a conformational exploration was carried out through accelerated molecular dynamics simulations (aMD). These simulations allowed us to obtain diverse conformational variants (inactive, intermediate and active), favoring the selection of small molecules compatible

with the receptor active state. Finally, we searched for potential agonists through virtual screening, applying an ensemble docking based strategy using multiple snapshots of GHSR1a-LR receptor extracted from aMD trajectories. A small molecule library, containing 4997 positive or neutral charged compounds obtained from ZINC12 database, was filtered under parameters of drug-like properties and possible central nervous system activity. From this campaign four agonist-potential molecules, based on predicted affinity in different receptor structural samples, were identified.

64. SYNAPTIC EFFECTS OF THE ALKALOID GELSEMINE ON CORTICAL NEURONS.

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Several behavior studies have suggested that the natural alkaloid gelsemine has different biological effect, such as analgesia and anxiolytic. Nevertheless, until now there is few information about neurophisyological mechanism, and pharmacological target associated to this biological effect. Early studies from our lab have shown that gelsemine decreases the frequency of glycinergic and glutamatergic events in the spinal cord neurons culture that suggest important effect in the synaptic function. Despite these advances, it is unknown if gelsemine can modulate GABAARs and GABAergic synapsis, which is relevant in the pathological anxiety phenomena. Here, we examined the functional effects of gelsemine on native sistem using electrophysiological techniques. Studies performed on cultured neurons was realized to explore the potential effect of gelsemine in the synaptic activity of cortical neurons, which express the functional component of a GABAergic synaptic. Our electrophysiological result show that gelsemine 50 mM produced a significative reduction of the frequency, but not in the amplitud of the GABAergic and glutamatergic synaptic activity. I addittion, Effects of gelsemine on the agonist sensitivity and on the desensitization rates of GABAAR native, are evaluated. Analysis of concentration – response curves revealed that gelsemine significantly decreased the apparent affinity for GABAAR without changing the maximal current amplitudes. Analyses of the GABA-activated currents stimulated by saturating agonist concentrations indicated that gelsemine did notmodify the fraction of desensitized current or the decay time constant of receptors. Our results that gelsemine is able to negatively modulate the synaptic activity of cortical neurons. Future studies may contribute to shed light on the mechanisms underlying the beneficial effects of the Gelsemium alkaloids in the control of pathological anxiety through the modulation of inhibitory receptors.

65. CYTOTOXIC EFFECT OF HYDROXYCHLOROQUINE, ITRACONAZOLE AND CISPLATIN ON SPHEROID CULTURE OF ORAL SQUAMOUS CELL CARCINOMA.

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Oral Squamous Cell Carcinoma (OSCC) is the most common type of oral cancer and Cisplatin is the chemotherapeutic agent most commonly used, which induces cell death by apoptosis. Furthermore, tumor cells (TC) acquire resistance against cytotoxic effect of the drug. Therefore, it is necessary to develop more effective treatments based on the metabolic changes that occur in TC. Hydroxychloroquine and Itraconazole are two drugs traditionally used in traditional medicine, as immunomodulator and antifungal, respectively. Recently, it has been described that they may have antitumor effect, because Hydroxychloroquine may inhibit autophagy, a mechanism of adaptation to metabolic stress, and Itraconazole would disturb energy metabolism. Tumor spheroid cultures are an in vitro model suitable for antitumor activity evaluation because they can reproduce tumors in vivo main characteristics, such as hypoxia-related drug resistance and the presence of Cancer Stem Cell (CSC), which may be responsible of chemotherapy resistance and tumor recurrence. In this project the expression of the markers of CSC CD44, CD56 and ALDHA1 in spheroidal cultures of Cal-27 (COCE cells) was evaluated through flow cytometry. The results show greater expression of these markers in spheroidal cultures, compared to monolayer cultures. In addition, cytotoxicity for spheroidal cultures was determined by the MTT assay for Hydroxychloroquine, Itraconazole and Cisplatin. The IC50s were 249, 472 and 577 micromolar at 48 hours and 272, 298 and 261 micromolar at 72 hours, respectively. A viability decrease induced by these drugs was observed in a concentration-dependent manner. At present is being evaluated if the drug combinations have

a synergy effect reducing the individually required concentration.

66. NEONATAL EXPOSURE TO TESTOSTERONE PROPIONATE INDUCES AN INCREASED EXPRESSION OF RGS9-2 AND PKCB2 IN NACC AND VTA OF ADULT FEMALE RATS.

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Research in programming is focused on the study of stimuli that alters sensitive periods in development, such as prenatal and neonatal stages, that can produce long-term deleterious effects in various organs or tissues such as the brain, affecting brain circuits and related behaviors. Previously, we have demonstrated that neonatal programming with sex hormones affects the mesocorticolimbic circuitry, increasing the synthesis and release of dopamine (DA) in striatum and Nucleus accumbens (NAcc); also, we have observed a reduction of locomotor activity in response to methylphenidate in female rats treated with testosterone propionate (TP). However, is not clear if the alterations observed in our model are related to modifications in the signaling pathway or DA release/uptake. Interestingly, RGS9-2, which is expressed in dopaminergic neurons, can inhibit the signal transduction of dopamine receptor 2 (D2) and have been related to drug addiction and movement disorders. Also, PKCβ2 can increase the amphetamine-stimulated dopamine efflux regulating the Dopamine Transporter (DAT) activity. The objective of this work was to evaluate if the neonatal reprogramming with Estradiol Valerate (EV) or TP affects the expression of Rgs9-2 and Pkcβ2 in NAcc and Ventral Tegmental Area (VTA) in adult rats using qPCR. The expression of Rgs9-2 and Pkcβ2 was increased in NAcc and VTA of female rats treated with TP; no significant changes were observed in males under any condition. These results suggest that the neonatal exposure to TP modifies the expression of Rgs9-2 and Pkcβ2 in female rats, and this modification can account for the modifications in response to methylphenidate observed in our model. Further analysis using WB or IHC are needed to depict the functional alteration in this model.

67. SIMVASTATIN AND 15-EPI-LIPOXIN A4 INDUCE CARDIAC REPAIR THROUGH NOTCH 1 ACTIVATION IN CHRONIC CHAGAS CARDIOMYOPATHY.

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Chagas Disease, caused by Trypanosoma cruzi, is endemic in Latin America and worldwide because of migration. Without appropriate treatment this disease progress to a chronic phase that could affects the heart. Chronic Chagas Cardiomyopathy (CCC), the most severe clinical manifestation, involves a progressive inflammatory myocarditis affecting ventricular wall causing cardiovascular complications due to diminished cardiac function and heart failure. Despite intense research no one drug can stop or reverse the progressive heart damage. Simvastatin, a drug that decreases blood cholesterol, has anti-inflammatory effects and inhibits platelet aggregation. Previously, we described that simvastatin reduces myocardial inflammation caused by T. cruzi through 15-epi-lipoxin A4 (15-epi-LXA4) production, a pro-resolutory inflammation molecule. Several reports suggest that simvastatin activates Notch pathway after a stroke enhancing blood flow by promoting angiogenesis. CCC progress with myocardial inflammation, endothelial damage with micro focal ischemia and fibrosis. We propose that simvastatin reverts cardiac damage in the chronic T. cruzi infection by 15-epi-LXA4 production and Notch 1 pathway activation. BALB/c mice were chronically infected with T. cruzi Dm28c strain and treated with simvastatin 1 mg/Kg/day and 15-epi-lipoxin A4 25 µg/Kg/day for 20 days. At day 80 post-infection animals were euthanized to analyze the heart, Notch pathway, fibrosis, and angiogenesis process. In chagasic mice, the cardiac function was restored with simvastatin and 15-epi-lipoxin A4 treatment. The Notch signaling pathway was active in cardiac tissue, a finding that correlated with drug treatment, the fibrosis process was decreased, and angiogenesis was also evidenced in this model. Thus, we concluded

that simvastatin and 15-epi-LXA4 improve cardiac architecture and function through Notch 1 activation by increasing blood flow and decreasing cardiac remodeling. Thus, it could be incorporated rapidly in CCC treatment.

68. INHIBITORY ACTIVITY OF ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE FROM AMARYLLIS BELLADONNA ALKALOIDS.

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Alzheimer's disease, a neurodegenerative disorder characterized by an irreversible and progressive loss of memory. Traditionally the pharmacological treatment associated with the disease in its medium to moderate stages, or similar diseases related to a deficit of the neurotransmitter acetylcholine are mainly guided through inhibitors of the enzyme acetylcholinesterase. Only four compounds have been approved as treatment against alzheimer diseases: donepezil, rivastigmine, galantamine and memantine. In most cases these drugs are well tolerated, however, various side effects such as: nausea, vomiting, among others may occur. That is why it is necessary to search for new molecules with pharmacological potential for their treatment. A potential source of acetylcholinesterase inhibitors is Amaryllis belladonna, belonging to the Amaryllidaceae family, widely distributed worldwide. Several studies support the presence of alkaloids in this species with varied biological activities. Considering the variation in the production of metabolites reported in this species, depending on the geographical distribution, it is interesting to analyze the alkaloids present in the representative of this family introduced in our country and its pharmacological potential. The objective of this research was to evaluate the inhibitory activity of alkaloids isolated from Amaryllis belladonna on enzymes acetylcholinesterase and butyrylcholinesterase.The alkaloids present in the plant's bulbs were obtained by maceration with methanol and subsequently fractionated, for the isolation. The putative alkaloid composition of the fraction was analyzed by GC-MS, highlighting the presence of type licorin and crinamine. The inhibitory activity of the alkaloid extract and the isolated compounds was evaluated by the Ellman method, finding IC50 values (ug / mL) for hexane, chloroform and butanol extract of 17.12, 8.89 and 19.09 for acetylcholinesterase and 77.27, 55.44 and 200 for butyrylcholinesterase respectively.

69. USE OF MEDICINES BY SOUTHERN BRAZIL FARMERS AND ITS RELATIONSHIP WITH EXPOSURE TO PESTICIDES FROM DIFFERENT CROPS.

Calinca Skonieski Karina Raquel Fagundes Matheus Henrique Machado Bento Anderson Joel Martino Andrade Daniel Barbosa de Chaves Samara de Cesaro Cavaler Andressa Talita Nunes Maiara Grasiela Rossi Fabiana Elias Dalila Moter Benvegnú. Universidade Federal da Fronteira Sul - Campus Realeza.

Agriculture in its development model has not been addressing issues such as the environment. Consequently, countless substances ended up being released into the environment, plenty of them with the ability to alter the behavior of several physiological systems, inducing numerous pathologies. Concomitantly, several studies have demonstrated a growing use of medicines. Therefore, the goal of this study is to investigate the relationship between the use of medicines by farmers and their exposure to pesticides. Upon approval by the Research Ethics Committee from the Federal University of the Southern Border, farmers were randomly selected in two cities: Mafra, Santa Catarina and Planalto, Paraná, both in Southern Brazil. The subjects were then asked to fill up a form for data collection. A total number of 251 farmers participated in the research, being 123 from Mafra and 128 from Planalto. The average age of the subjects in this study was 48,4 ± 14,4 and 114 were female while 137 were male. Out of these, 23,1% (58) are making use of neuropsychiatric drugs, 32,7% (82) of cardiovascular drugs, 17,5% (44) of metabolic disorder drugs, 0,8% (2) of respiratory disorder drugs, 1,6% (4) of gastrointestinal drugs and 2% (5) of musculoskeletal purposes drugs. When correlation tests were performed between the type of crops and the drugs used by the respective farmers, the results showed a greater use of medicines for metabolic ($p = 0.014$) and musculoskeletal disorders ($p =$

0.025) from wheat crops farmers in Mafra. Thus, the data suggests that farmers in wheat crops are more likely to make use of drugs for metabolism and musculoskeletal disorders, as it might be related to the specific pesticides used in this crop.

70. SUCRALOSE INTAKE IMPAIR THE HIPPOCAMPAL POSTSYNAPTIC INHIBITORY CURRENTS.

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Non-caloric sweeteners (NCS) are widely used in foods with the aim of reducing sugar consumption and caloric intake. Sucralose is the most used NCS worldwide and in Chile, with the current "Labeling Law", its consumption has been increasing. Sucralose intake causes alterations in the composition of the intestinal microbiota, which is closely related to mental health through the crosstalk communication between gut and brain. Considering that microbiota can affect behavior and modulate GABA levels, brain plasticity and cognitive function, we wonder whether NCS affect the integration and synaptic function in hippocampal CA1 pyramidal neurons in adult Sprague Dawley rats treated with 0.5% sucralose in the drinking water for a period exceeding 17 days. Using patch-clamp recordings in whole configuration we observe that membrane potential pyramidal neurons NCS treated rats have a more depolarized value than control group and no effect on the trigger threshold of action potentials. Also, we observe that frequency of the spontaneous inhibitory synaptic currents in PYNs is lower than control slices. The paired pulse protocol did not show differences between animals treated with NCS and control, suggesting that NCS intake modify the presynaptic and postsynaptic excitability, no apparent effect on the release of GABA. These results show for the first time that permanent consumption of sucralose may have affect GABAergic synaptic efficacy in the central nervous system.

71. ANTITUMOR PROTOTYPE BASED ON POLYMERIC NANOPARTICLES WITH APPLICATION IN GENE THERAPY.

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Gene therapy is a therapeutic strategy mainly focused on correcting altered or mutated genetic sequences, which can induce the development of hereditary or acquired pathologies, such as cancer. Nanoparticles based on biocompatible polymers have been used as carriers for therapeutic molecules, due to their ability to encapsulate labile molecules such as linear or plasmidial DNA by electrostatic interactions. Thus, it could prevent their degradation by nucleases present in the environment. Safety and effectiveness of the polymers make feasible in vivo tests and future commercial products as described and approved by regulatory agencies, such as the FDA. The aim of this work was to design and evaluate a formulation based on polymeric nanoparticles as gene therapy applied to prostate cancer. Nanoparticles were elaborated using a double emulsion method, with solvent evaporation, and PLGA as the main matrix agent, associated with a cationic polymer. A model plasmid, which transcribes a tumor progression blocker was encapsulated. Physicochemical characteristics of nanoparticles were analyzed by Zetasizer Nano, and their effect was evaluated in vitro, in a human tumor cell line. Nanoparticles were obtained in nanometric size range, with a polydispersion index (PdI) less than 0.2, and the surface charge was positive. Morphologically, nanoparticles were spherical according to Transmission Electron Microscope images. It was also observed an increase in the genetic transformation rate for human tumor cells, and an alteration in the characteristic tumor progression markers expression. These results are promising for the development of new therapeutic candidates based on nano delivery system for complementary treatment in different types of cancer.

72. RESOLVIN D1 PREVENTS CARDIAC HYPERTROPHY AND FIBROSIS IN ANGIOTENSIN II-INFUSED C57BL/6 MICE.

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1,2
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Background: Resolvin D1 (RvD1) is an endogenous specialized lipid mediator enzymatically derived from docosahexaenoic acid and synthesized locally in acute inflammatory processes, where it exerts pro-resolving effects demonstrated in diverse pathological models. Angiotensin II (Ang II), in cardiac tissue, contributes to the development of cardiac hypertrophy and fibrosis. To date, there are not studies on the potential protection that RvD1 may provide at the structural and functional level in an Ang-II infusion model. Purpose: To evaluate RvD1 effects in Ang II-induced cardiac hypertrophy and fibrosis. Methods: Alzet® osmotic mini-pumps filled with Ang II (1.5 mg/kg/day) were implanted in C57BL/6 mice for 14 days, previous basal left ventricle (LV) functionality assessment. RvD1 (3 ug/day) was injected intraperitoneally. At the end of the infusion period, the animals were sacrificed, and functional and histological parameters were studied. Results: 14-day Ang II infusion increased heart weight/tibia length ratio, LV thickness, ejection fraction, shortening fraction and collagen deposition at the interstitial and perivascular area. Treatment with RvD1 significantly prevented LV dysfunction, hypertrophy and collagen deposition in both areas. Conclusions: RvD1 prevents Ang II-induced cardiac hypertrophy and fibrosis demonstrating cardioprotective properties. Further studies will be performed to elucidate the possible mechanisms of action of RvD1. Ethics approval: The Institutional Animal Care and Use Committee of the University of Chile (CICUA) approved the protocol (CBE2018-12).

73. ANTIBIOTIC SUSCEPTIBILITY PROFILE OF HELICOBACTER PYLORI IN THE ARAUCANÍA REGION.

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Introduction: Antibiotic resistance is one of the main causes of therapeutic failure in eradication treatments of Helicobacter pylori (Hp), which currently and according to Maastricht consensus the standard consists of a triple scheme of a proton-pump inhibitors (PPI) +2 antibiotics. In recent years, high resistance rates to the main antibiotics used in these treatments have been reported in several countries. Even varying between geographical areas of the same country, which prevents generalizing efficient therapies in certain populations without previous susceptibility studies Aim: To evaluate the susceptibility profile of Hp, in the Araucania region, against antibiotics used in eradication therapy. Methods: A descriptive study on 37 Hp, isolates from gastric biopsy samples on dyspeptic patients was performed in main health centers of the Araucania. The susceptibility profile against amoxicillin, clarithromycin, levofloxacin, metronidazole and tetracycline was performed by agar dilution. Minimum inhibitory concentration values were evaluated according European Committee on Antimicrobial Susceptibility Testing, using Hp ATCC 43504 as a quality control strain. Results: All isolates reported resistance at least one antibiotic and 81.08% showed resistance to two or more antibiotics. 13.8% of the Hp isolates were resistant to amoxicillin, 45.94% to clarithromycin, 41.66% to levofloxacine, 81.08% to metronidazole and 16.66% to tetracycline. Conclusion: The resistance rates to metronidazole, clarothromycin and amoxicillin were higher to reported in Chile and there are not previous reports to LVZ. These results show the need of future studies of therapeutic efficacy in the Araucanía as well a new review of current eradication strategies.

74. ANALYSIS OF THE FUNCTIONAL SPECIFICITY IN THE SUGAR PORTER FAMILY TO IDENTIFY NEW INHIBITORS OF GLUT1.

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The movement of glucose and other sugars and polyols with essential functions in the energy metabolism of living beings through biological membranes is carried out by transporter proteins belonging to the family of sugar transporters (SP family, TC code 2.A.1.1), where we find the human GLUT transporters, the hexoses transporters in yeasts, among others. There are highly specific transporters for a physiological substrate, and there are others much more promiscuous. There is an increasing interest in pharmacology to design GLUTs inhibitors, whose development has risen since the determination of the crystallographic structure of human GLUT1. For glucose homeostasis diseases that occur with hyperglycemia and cancer, the therapeutic use of these molecules has been proposed. In the first case, its potential use is in the downregulation of the incorporation of glucose to the blood, while in cancer the aim is to avoid the dispensation of glucose to cells with active proliferation. Several inhibitors of transporters are designed from substrate/solute modifications. To advance in the understanding of the molecular bases that explain the varied selectivity of the SP family, we carried out the present work, which consists of the identification and characterization of sequence profiles related to the specificity of substrates for the SP family. To achieve this goal, we first characterized the functional diversity of the family by identifying functional subclasses using supervised methods that build the functional classification based on empirically obtained transport activities reported for the transporters from databases. From the analysis of the specificity determinant positions (SDPs), we build structures to find inhibitors in a pharmacophore-like mode to accelerate the identification of new inhibitors and predict the selectivity of other GLUT1 orthologues.

75. INHIBITORY ACTIVITY ON GLYCOGEN PHOSPHORYLASE A OF PHENOLIC EXTRACTS FROM LEAVES AND FRUITS OF 8 UGNI MOLINAE TURCZ GENOTYPES.

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One of the strategies used for the discovery of natural products with hypoglycemic activity is the analysis of species used by traditional medicine, such as Ugni molinae, Myrtaceae, popularly known as murtilla. In this context, the analysis of the inhibition on the activity of Glycogen phosphorylase A (Gpa), an enzyme expressed in brain, muscle and liver, which has major role on post-prandial hyperglycemic peaks in diabetic patients, could account for potential candidates for the development of new treatments. Therefore, the aim of this work was to demonstrate and compare, through an in vitro spectrophotometric methodology, the Gpa inhibitory activity of phenolic-rich extracts obtained from leaves and fruits of 8 murtilla genotypes from the INIA-Carillanca germplasm bank, which were cultivated at the same edaphoclimatic conditions. Compared to caffeine (IC50 = 5,3 ug / mL), the leaves ethanolic extracts were more potent (EETs; IC50 between 1,03 – 3,52 ug / mL; $p \leq 0,05$), while the fruit acetonic extracts were less potent (EACs; IC50 between 27,9 – 86,1 ug / mL; $p \leq 0,05$) than the reference substance, as well as less potent than the leaves extracts. Based on our results, the leaves and fruits of U. molinae could be a potential source of bioactive phenolic compounds for the treatment of hyperglycemia, through the development of functional foods or phytopharmaceuticals. On the other hand, based on the results from this work, INIA-Carillanca will be able to classify the genotypes for their potential hypoglycemic effects, which will allow the future to promote the cultivation of murtilla for its agronomic and commercial value, as well as for its medicinal properties.

76. EVALUATION OF ANTICANCER POTENTIAL OF DHA + P1G10 IN CELL LINES DERIVED FROM CANCERS WITH HIGH INCIDENCE IN CHILE.

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Cancer is one of the most frequently diagnosed diseases worldwide, being the second most frequent cause of death. Previous studies have established that omega-3 fatty acids, mainly DHA (docosahexaenoic acid), have protective effects against various types of cancer, among these, gastric cancer, which is one of the most common cancers in the world, with one of the highest mortality rates in Chile. On the other hand, Carica papaya protein fraction P1G10 also has proven anti-cancer properties due to its proteinase activity. Our data indicate that gastric adenocarcinoma cells (AGS) are more sensitive to DHA than non-tumor gastric epithelium cells (GES-1), determining, through MTT, an IC50 of 40.47 μM in AGS. Through Hoechst/Annexin V/IP was found that DHA promotes apoptosis in AGS cells, but not in GES-1. It was also determined that DHA decrease procaspase-3 protein levels in AGS cells only. In vivo assays in BALB/c NOD/Scid mice conclude that DHA treatment for 6 weeks significantly decreases the volumes of tumors generated by AGS cells xenografts. To assess the effects of DHA+P1G10 in vitro, we determined cell proliferation through MTT, treating cell lines derived from the main types of cancer in our country: gastric, lung, gallbladder and breast. To observe cell apoptosis/necrosis visually, we will stain treated cells with Hoechst/Annexin V/PI solution, and to gain further insight into the mechanism of DHA+P1G10 -induced apoptosis, we will examine protein levels of procaspase-3 and caspase-3/7 activity using Western blot and luminescence assay, respectively. Thus, this research seeks to determine and validate the joint use of DHA and the protein fraction P1G10, on cell lines of the main types of cancer in Chile.

77. ANXIOGENIC EFFECT OF AMPHETAMINE ON ZEBRAFISH USING A NOVEL TANK DIVING TEST AND MONOAMINE TRANSPORTER GENES EXPRESSION.

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Monoamine Transporters regulate neurotransmission via the reuptake of dopamine, serotonin and norepinephrine in the brain and regulate the neurotransmitters homeostasis. This class of protein are target for a wide number of compounds including antidepressants, drugs for neuropsychiatric and neurodegenerative disorders, and substance of abuse, such as amphetamine. This drug of abuse has been described that produce anxiogenic effect on rodents and it is well known that interacts with monoamine transporters inducing monoaminergic release. In our group we have used zebrafish as models for behaviour using different drugs that acts over nicotinic receptors and monoamine transporters. The novel tank diving test has been used as a model for to test anxiolytic behaviour on zebrafish, the time spending on the bottom of the tank has been describe as anxiogenic-like behaviour in this model. This work shows the anxiogenic-like effects produced by amphetamine on the novel tank diving test. Additionally, we design the primers and detect the genes expression of Crebs, DAT, NET and SERTa, SERTb by PCR. Also we measure the expression changes using qPCR for this monoamine transporter using a chronic dose of amphetamine. Our results indicate that, amphetamine induce anxiogenics-like effects of diving behaviour and increase genes expression of MAT´s at different level.

78. MITOCHONDRIOTROPHIC POLIHYDROXY-BENZOATES DERIVATIVES AGENTS MODIFY THE EXPRESSION OF METASTATIC BIOMARKERS IN HUMAN COLORECTAL METASTATIC CELLS IN VITRO.

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The metabolic plasticity of cancer cells is the main limiting factor in the research of effective pharmacologic treatments, for development of drug resistance, being one of the major obstacles in the clinical treatment, as a promising target for new anti-cancer drugs therapies. Mitochondria have been the main factor in the metabolic plasticity. This organelle also participates promoting metastasis, tumor-initiating cells and survival and propagation of cancer stem cells, which transforms it into an attractive therapeutic target. Previously reports have demonstrated that GA-TPP+C10 triggered a mitochondrial dysfunction, characterized by an inhibition of electron transport chain (ETC) and AKGDH complex inhibition which triggers cell death. In order to increase this effect, we have analyzed mitochondrial resistance mechanism generated by the action of this compound, highlighting an increased expression of PGC1α and ETC components-related genes encoded by mitochondrial DNA. In order to inhibit the resistance generated by this inhibitory mechanism of action, we have incorporated a second agent, doxycycline, which demonstrated inhibits the synthesis mitochondrial proteins by blockage of only mt-ribosome activity. This effect inhibits the adaptive survival response generated to the action of GA-TPP+C10 evidenced by inhibition of ETC-related protein. Interestingly, the combined therapy increments significatively the mRNA levels, both ETC-components and mitochondrial biogenesis signaling factors (PGC1a-TFAM-NRF1-NRF2), which suggests a greater mitochondrial damage, evidenced by a decreased mitochondrial mass with a consequent decreased of the maximal respiration. In addition, concomitant use of GA-TPP+C10 and doxycycline is able to generate a selective synergic cytotoxic effect on the activation of apoptotic processes in BC cells, which suggest that this combined strategy based on the blockage of mitochondrial bioenergetics inhibition-induced adaptive response may have therapeutic relevance in breast cancer.

79. AMYLOID BETA OLIGOMERS INDUCE MITOCHONDRIAL DYSFUNCTION BY ITS DIRECT INTERACTION WITH MITOCHONDRIAL MEMBRANES ON HIPPOCAMPAL SLICES.

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by impaired learning and memory loss. Amyloid beta peptide (Aβ) plays a key role in the pathogenesis of AD, especially soluble oligomers (SO-Aβ) because can reproduce the major aspects of the disease. In vitro studies have associated mitochondrial dysfunction with an early role in the AD; however, the molecular events are not understood with precision. In this work, we have studied the intracellular effects of SO-Aβ treatments, on mitochondrial morphology and mitochondrial potential (ΔΨm). We found that the degree of colocalization between Aβ and TOM20 was increasing at 24 h of SO-Aβ treatments, with a Manders coefficient (0.640 ± 0.1). Furthermore, we evaluated the ΔΨm using the JC-1 probe, we observed that at chronic treatments (24h), SO-Aβ shown a decrease on ΔΨm near to 50% of the control conditions. Additionally, at the same times (SO-Aβ, 24h) strong changes were observed in the size of the mitochondrial network in primary cultures, displacing the equilibrium towards a more granular pattern in mitochondria that present a positive colocalization with Aβ. Secondly, the intracellular distribution of SO-Aβ (2.5uM) in a mouse hippocampal slices model was evaluated by immunohistochemistry and electron transmission microscopy (TEM), where we observed the presence Aβ targeted with gold nanoparticles in an intramitochondrial zone. On the other hand, it was observed that ΔΨm showed

a progressive decrease in time manner on under SO-Aβ treatments (JC-1590/520 C: 1.01 ± 0.01; SO-Aβ 3h: 0.78 ± 0.04). This study suggest a new pathogenic mechanism in AD, where cytotoxic effects of SO-Aβ are related with their direct interaction with the mitochondria, and reveals a novel therapeutic strategies for neuroprotection.

80. CYTOTOXIC EFFECT OF COMBINATIONS OF ITRACONAZOLE, HYDROXYCHLOROQUINE AND CISPLATIN IN HEAD AND NECK CARCINOMA IN LOW GLUCOSE CULTURES.

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Head and neck cancer (HNC) is the sixth most common malignancy in the world, corresponds to 6% of cancer cases and is responsible for 1-2% of deaths worldwide. The most prevalent HNC subtypes are laryngeal cancer and oral squamous cell carcinoma. These pathologies are very aggressive and have poor prognosis and recurrences, which can be caused by a possible resistance to chemotherapy. Cisplatin is the chemotherapeutic most used to treat these pathologies, however it has been high rates of resistance. This resistance may be caused partially by "Cancer stem cells", which are resistant to stress stimuli such as starvation and low oxygen levels. It has been described in some studies that there are drugs, such as Itraconazole and hydroxychloroquine, an antifungal drug that acts at the mitochondrial membrane of the tumor cell by inhibiting the VDAC1 receptor, and an antimalarial/ immunosuppressive that has been described with antineoplastic potential by inhibiting autophagy, respectively. In this way it is proposed that the combination of these drugs sensitize the effect of Cisplatin. Cell viability tests were performed with the compounds at 24, 48 and 72 hours under normoxia conditions with low glucose medium (1,0 g/L) in two cell lines, laryngeal squamous cell carcinoma (HEp-2) and squamous tongue carcinoma (CAL-27), using as a control oral dysplastic cells (DOK). This will be carried out in order to obtain the IC50 of each compound and determine their cytotoxic effect. A cytotoxic effect has been observed for all compounds assessed on tumor cells, highlighting the efficacy of Hydroxychloroquine over Cisplatin and Itraconazole. The combination of hydroxychloroquine or itraconazole with cisplatin, improve the cytotoxic effects on tumor cells.

81. ANTIMICROBIAL SUSCEPTIBILITY TESTS OF HELICOBACTER PYLORI ISOLATES FROM PATIENTS IN THE BIOBÍO REGION: COMPARISON OF AGAR DILUTION AND DISK DIFFUSION.

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Antimicrobial susceptibility testing for Helicobacter pylori is increasingly important due to resistance to the most commonly used antimicrobial agents. The Gold Standard proposed by the CLSI is the agar dilution method, but it is difficult to perform routinely. The objective of this work was to determine the concordance of disc diffusion in comparison to the agar dilution method for: clarithromycin, metronidazole, levofloxacin, amoxicillin and tetracycline, using 44 strains of H. pylori from patients in the BioBío region. Univariate analysis was performed, and the Kappa test was applied for concordance using the Stata V.14 program. The resistance rates were for clarithromycin 29.5% and 25.0%; metronidazole 45.4% and 56.8%; Levofloxacin 31.8% and 25.0; Amoxicillin 2.2% and 0% respectively by disk diffusion and agar dilution. Tetracycline showed no resistance with any of the 2 methods used. Clarithromycin presented a considerable degree of concordance with a k = 0.6571 (p <0.0001). Metronidazole did not show concordance for the techniques under study (p=0.1586). Levofloxacin presented an almost perfect concordance with a k=0.8333 (p<0.0001). On the other hand, the Kappa test was not calculated for amoxicillin and tetracycline, since 97.3% and 100% concordance were obtained respectively. The disc diffusion method presented a high degree of agreement with the Gold Standard for clarithromycin, levofloxacin, amoxicillin and tetracycline. This is an easy method to assess susceptibility to H. pylori especially if it is performed routinely. For metronidazole there was a high degree of disagreement with agar dilution, which has already been reported. Finally, other studies with a greater number of isolations are necessary to assess whether the method of disk diffusion, which is simpler and cheaper, can be continued routinely in our region.

82. POLYMERIC BIOCOMPATIBLE NANOCARRIERS FOR DRUG DELIVERY APPLICATIONS SHOWS PRESERVED BIOLOGICAL ACTIVITY OF LOADED PROTEINS, IN VITRO AND IN VIVO.

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In the search for new and more effective therapies, polymeric nanoparticulate systems which protect the drugs and increase bioavailability have been developed. The use of biocompatible and biodegradable polymers could guarantee the harmless character of the formulation while allows the controlled release of the active principle. Using ionotropic gelation method, we synthesized chitosan-TPP nanoparticles loading recombinant and model proteins, in a reproducible way. This nanoparticulate system showed a peak of protein released around the fourth day, in vitro, and promoted the internalization of loaded BSA-FITC conjugates by Hep-2 cells after 24 hours of incubation. Cytotoxicity assay evidenced the benign character of the formulation, while experiments of biological activity in vitro and in vivo, showed a specific biological response due to the system loading. Visualization of the nanoparticles was possible thanks to transmission electronic microscopy. This procedure proved to be an effective method to formulate proteins, and, potentially, other molecules, in a safe way, while the release of the active principle can be delayed over time. This type of systems can be used in drug delivery applications in which pharmacological interaction with the cell is required.

83. LEUKEMIA INHIBITORY FACTOR, A NEW MODULATOR OF THE OVARIAN CHOLINERGIC SYSTEM IN SUBFERTILE RAT.

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Leukemia Inhibitory Factor (LIF) is a proinflammatory cytokine that participates the regulation of ovarian functions. LIF participation in the subfertility period has not been described and the mechanism of action is unknown. In vitro studies have shown that LIF increase the acetylcholine (ACh) synthesis, choline acetyltransferase (ChAT) expression and its activity in the upper cervical ganglion. In our laboratory, an intrinsic ovarian cholinergic system has been determined recently, which participates in the regulation of ovarian function. The aim was to evaluate the LIF/LIF Receptor (LIFR) levels and its effect on the ovarian cholinergic system in subfertile rats. We measured LIF and LIFR mRNA and protein levels by qRT-PCR and western-blot at 3 (fertile) and 9 months old (subfertile) Sprague-Dawley rats. To evaluate the LIF effect on the ovarian cholinergic system, rat ovaries were incubated in vitro for 3 and 8 h with LIF (100ng/ml) and buffer Krebs (vehicle). ACh production and the mRNA content of the genes encoding the ChAT and AChE enzymes it was determined by fluorometry and qRT-PCR respectively. The results show increase in LIF protein levels and increase of LIFR mRNA in ovaries in fertile period in subfertil period. Incubation with LIF increases ACh in incubation medium, without observing changes in ovarian ACh levels. The ChAT and AChE mRNA content enzymes significantly decrease at 3h of incubation (40% and 50%, respectively). In contrast, in ovaries incubated for 8 h, LIF does not affect ovarian ACh levels nor in the incubation medium. These results suggest that LIF regulates ovarian cholinergic function during reproductive aging, pending as LIF and the cholinergic system regulate follicular development at this period.

84. IDENTIFICATION OF RESIDUES INVOLVED IN THE DOPAMINE TRANSPORTER-GBETAGAMMA PHYSICAL/FUNCTIONAL INTERACTION.

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The dopamine transporter (DAT) plays a crucial role in the regulation of brain dopamine (DA) homeostasis. Through re-uptake of DA, DAT serves two important functions: the termination of synaptic transmission at dopaminergic terminals, and the replenishment of vesicular DA pools. In addition to uptake, DAT can also function to release DA. This process, which is referred to as DAT-mediated efflux, is the mechanism used by potent and highly addictive psychostimulants, such as amphetamine and its analogues, to increase extracellular DA levels in motivational and reward areas of the brain. It has long being recognized that DA neurons release DA through exocytotic and non-exocytotic processes. However, the exact mechanism by which physiological signals or psychostimulants, such as amphetamine, induce DA efflux through DAT still remains a complex and not completely understood area of research. Recently, we discovered that the G protein betagamma subunits bind to the intracellular carboxy-terminus of DAT and regulate transporter activity. More importantly, we have observed that activation of Gbetagamma promotes DAT-mediated DA efflux. However, the amino acid residues involved in Gbetagamma interaction site(s) in DAT and their role in transporter regulation remain largely unknown. Here, we used a combination of bioinformatics, mutagenesis, immunoprecipitations, and functional assays to identify the Gbetagamma binding site on DAT and its role in transporter regulation. Preliminary functional studies are consistent with previous biochemical evidence indicating that the sequence FREKL located in the carboxy-terminus of DAT plays a role in Gbetagamma interaction with DAT and promotion of DA efflux. Thus, this study provides a starting point for a further detailed characterization of the DAT-Gbetagamma interaction and a better understanding of its contribution to DAT-mediated efflux.

85. AMYLOID BETA OLIGOMERS INTERRUPT NUCLEAR CA2+ TRANSIENTS AND GENE EXPRESSION INDUCED BY GABAZINE IN HIPPOCAMPAL NEURONS.

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Ca2+ signals are essential mechanisms that regulate neuronal plasticity. Nuclear Ca2+ transients generated by neuronal activity induce changes in gene expression and in dendritic spine remodeling, which are mediated by the rapid activation and expression of transcription factors. Among them, Npas4 is known for inducing distinct activity-dependent gene programs that regulate the expression of neurotrophic factors and antioxidant enzymes. Thus, Npas4 may provide a molecular link between neuronal activity and the activation of memory and neuroprotection signaling pathways. Amyloid-beta oligomers (A-beta Oligomers) are synaptotoxins that induce aberrant Ca2+ signals and promote Reactive Oxygen Species (ROS) generation,leading to synaptic plasticity disruption. In this work, we studied the effects of AβOs in nuclear Ca2+ signals production and gene expression induced by Gabazine, a GABA(A) receptor blocker that functions as an inductor of synaptic activity. To this aim, we transfected primary hippocampal neuronal cultures with a genetically encoded Ca2+ indicator with nuclear destination (GCaMP3-NLS). We pre-incubated these cultures with AβOs for 6 h and applied Gabazine at the microscope stage, to record nuclear Ca2+ signals by live-imaging. We also performed immunocytochemistry to evaluate CREB phosphorylation and RT-qPCR to evaluate the mRNA expression of Npas4, BDNF and of the antioxidant enzymes

Glutamate-Cysteine-Ligase (GCL) and NADPH-Quinone-Oxidoreductase (Nqo1) in these conditions. Our results indicate that neurons treated with A-beta Oligomers showed reduced nuclear Ca2+signals and diminished Npas4, GCL and Nqo1 mRNA expression levels in response to GBZ. In summary, the present results indicate that A-beta Oligomers altered the activation of signaling pathways induced by gabazine, leading to a disruption of neuroprotective gene expression pathways essential to memory and learning processes, which are affected in neurodegenerative diseases.

86. EVALUATION OF SINAPTIC COMPONENTS DURING NEURULATION OF XENOPUS LAEVIS EMBRYOS.

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In chordates, neurulation and neural tube formation is the first step in the central nervous system (CNS) development. Failures, by genetic or environmental alterations, in this process may induce neural tube defects (NTDs). It has been described in the literature, that the use of anti-epileptic drugs (AEDs) during pregnancy, increase the probability of NTDs by mechanisms not completely identified. Recently, glutamate signaling through NMDAR has been proposed to participate in neurulation process. Here we hypothesized that glutamate would be released by a vesicular-related mechanisms which contribute to cellular responses necessary for normal neural tube formation. For evaluate this, we use Xenopus laevis embryos, collected in different neurulation stages (9 - 20 hours post-fertilization (hpf)) for obtain RNA transcripts. Then we performed PCR and qPCR for assess relative expression studies, focalized in evaluate the presence of vesicular release-related and synaptic receptor proteins. We observe the presence of vesicle related proteins, such as SNAP25, VAMP2, Syntaxin and VGLUT1, as well as glutamate receptor MGLuR2 and AMPAR during neurulation. Then, to evaluate the functionality of AMPAR in neurulation, we perform pharmacological studies, using the antagonist CNQX. We observe that CNQX don't provoke any evident alteration in neural tube formation. Finally, we performed an induction of epileptogenic behavior using Pentylenetetrazol to evaluate CNS health after neurula-treatments. We observe a decrease of almost ~50% in the seizure latency onset necessary for epileptogenic behavior vs not treated controls. Our results suggest that, glutamate could be released using vesicles proteins and the expression of AMPAR don't participate in the normal neural tube development but could regulate additional later process important for the CNS establishment on Xenopus laevis.

87. TRIPANOCIDAL ACTIVITY OF CASTANEDIA SANTAMARTENSIS (ASTERACEAE) AGAINST TRYPANOSOMA CRUZI.

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Chagas disease (CD) an endemic disease from Latin America, is caused by Trypanosoma cruzi infection. More than 7 million people are infected. Currently, there are two drugs derived from nitro compounds for the treatment of CD with important side effects, that cause the treatment to be abandoned, furthermore, the effectiveness in the chronic phase is still controversial. Therefore, is necessary the search for new drugs that are more effective and better tolerated. Castanedia santamartensis R. M. King & H. Rob, is known for their properties to treat skin sores. The objective of this study was to evaluate the trypanocidal activity of an ethanolic extract (ETE) of C. santamartensis and its fractions. Air-dried and powdered leaves, were extracted at room temperature with ethanol and concentrated and dried by evaporation at reduced pressure. The fractionation was obtained by chromatographic separation methods, using solvents of different polarity. The in vitro trypanocidal activity of the ETE and the fractions was determined against T. cruzi trypomastigotes (Dm28 strain)

using MTT and flow cytometry techniques. Nifurtimox was used as a reference drug. The IC50 (concentration that produce a 50% parasitic death) was calculated using the least squares method. The ETE presented trypanocidal activity (IC50 of 197.3 micrograms/mL). The CS200; CS300 and CS400 fractions, presented trypanocidal activity with IC50 values of 91.2; 63.9 and 15,4 micrograms/mL respectively. The IC50 of the reference drug was 5.7 micrograms/mL. The results indicate that C. santamartensis contains secondary metabolites with activity against T. cruzi.

88. EFFECTS OF P2X2R OVEREXPRESSION IN CELL LINES AND ITS IMPACT IN SIGNALING PATHWAYS ASSOCIATED TO AMPK/ CAMKII.

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One of the main toxic agents in Alzheimer's Disease (AD) are the soluble oligomers of the Aß peptide (OS-Aß). Chronic treatments with OS-Aß have been shown to increase the expression of the P2X2 receptor (P2X2R) in PC12 cells and rat hippocampal cells, participating in increasing intracellular calcium and allowing a leak of ATP to the extracellular environment. AMPK protein kinase has several roles on protein, energy and mitochondrial metabolism and is regulated by changes in the levels of intracellular Ca2+ and AMP/ATP ratio. AMPK is capable of phosphorylate PGC-1a, which is a transcription co-activator that, when is phosphorylated, is activated and translocates to the nucleus, promoting mitochondrial biogenesis. Using PC12 cells to overexpress P2X2R, the effect of its activation was assessed by ATP treatments, on AMPK activity and the subcellular distribution of PGC-1a. From functional experiments (calcium microfluorimetry and electrophysiology), immunocytochemistry and Western blot, it was concluded that overexpression and activation of P2X2R by ATP, prevents an increase in AMPK activity and generates changes in the subcellular distribution of PGC-1a, which suggests that P2X2R would be related to the toxicity generated by the Aß peptide and the intracellular calcium overload.

89. NEW LIPOPHILIC CATIONS DERIVED FROM CAFFEIC ACID INDUCE CYTOTOXIC EFFECT IN HUMAN COLORECTAL CANCER CELLS.

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One of the deadliest pathologies worldwide is cancer. Colorectal cancer is the third most common type of cancer. A few drugs are provided for treatment this disease, like 5-fluorouracil, oxaliplatin and irinotecan, as standard therapy. However, this therapy several times failed due to high drug resistance and side effects, leading cancer progression. There are several risk factors both exogenous and endogenous that increase the incidence of this disease in the organism, hence the importance of characterize the cancer cells from cytological, metabolic and molecular aspects. These features give them the differences between normal epithelium cells, becoming with high proliferative rates in an uncontrolled manner. In this sense, the mitochondria appear as a new target for new molecules against cancer, since they have high mitochondrial-transmembrane potential than normal cells, capable to accumulate cationic compounds. This work is focused in the evaluation of a new set of molecules derivatives from caffeic acid attached to a different size length of triphenylphosphonium-aliphatic chain and their effect on human colorectal cancer cells. We evaluated cytotoxic effect by MTT assay, the decrease of mitochondrial potential by flow cytometry and the decrease of cellular of ATP levels by luminescence. The results showed that the compounds were cytotoxic in colorectal cell lines (HCT-15 and COLO 205), decreasing mitochondrial-transmembrane potential and cellular ATP levels. In conclusion, these new compounds may induce cytotoxic effect by a mitochondrial mechanism, inducing bioenergetics stress, suggesting the importance of studying new pharmacological agents taking advantage of the cellular singularities like mitochondrial metabolism.

90. BIOLOGICAL EFFECT OF NOVEL TRIAZOLOPYRIDINES AGAINST MACROPHAGES MURINE.

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Recently, in our group we have synthesized and characterized triazolo pyridine derivatives, based on a rational study (structure-activity), where we found that compound 1 has an antiproliferative effect against the replicative form of the Trypanosome cruzi parasite. However, to evaluate the cytotoxic activity of the new [1.2.3] triazolo [1.5a] pyridine in a murine cell model, which is the first defense system against T. cruzi, we find the macrophages. Viability results by MTT indicate that compound 1 and 2, have cytotoxic activity in this system. On the other hand, by flow cytometry and propidium iodide, we have found that compound 2 can stop the cell cycle, consequently, stop cell proliferation and induce apoptosis death processes. This effect is observed in the literature with classic sterols synthesis inhibitors. This indicates that they are possibly affecting an enzyme of the p450 complex in mammalian cells.

91. P2Y2R AND P2X4R EXPRESSION PROFILE AND ITS ROLE IN PROLIFERATION AND METASTATIC POTENTIAL IN GASTRIC CANCER CELL LINES.

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Gastric cancer is considered a major health concern due to its unspecific symptomatology on early stages and complex pathophysiology that hinders any attempts for targeted therapeutic approaches. This disease has high incidence and mortality rates worldwide, being the third cancer-related cause of death in Chile, which focuses scientific work in understanding the mechanisms that trigger abnormal proliferation rates and subsequent tumor migration in gastric epithelia. Between these possibilities, purinergic signaling emerges as promising pathway that regulate cell growth, proliferation and migration according to the expression rates of its many receptor classes and subclasses. Among these, P2Y2 receptor (P2Y2R) is widely known by its contribution to cell invasion and metastasis in prostate, colorectal and colon cancer; which is in contrast to the antiproliferative effects reported for P2X4 receptor (P2X4R) on cancer models in vitro. Despite all this background, purinergic signaling involvement in gastric cancer remains unknown. For this reason, our investigation was focused to characterize the expression profile of P2Y2R and P2X4R, in terms of protein levels by western blot and gene expression by qPCR, in cell lines derived from primary gastric adenocarcinoma (AGS), moderately and mildly differentiated metastatic gastric adenocarcinoma (MKN-74 and MKN-45, respectively) and healthy gastric epithelia (GES-1). Moreover, to assess P2Y2R and P2X4R contribution to gastric cancer growth and invasion, we evaluated the effect of different agonists and antagonists on cell proliferation by Resazurin assay, and stablished metastatic potential by transepithelial electrical resistance (TEER) measurements in the gastric cell lines described above after overexpressing or silencing P2Y2R and P2X4R. Our results provide preliminary insights on gastric cancer pathophysiology that can be used as future pharmacological approaches for treatment.

92. INTRACELLULAR AMYLOID-BETA OLIGOMERS DECREASE EXCITABILITY AND AMPA MEDIATED CURRENT IN NUCLEUS ACCUMBENS NEURONS.

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Alzheimer disease (AD) is a progressive neurological disorder that causes dementia in an increasingly aging worldwide population. Despite the current dogma of AD, where extracellular aggregates of amyloid beta peptide (Aß) initiates neurotoxicity, growing evidence shows synaptic dysfunction and loss of limbic functions in early stages before amyloid plaque deposition, where the presence of intracellular Aß has been reported. The nucleus accumbens (NAc), a central integrative brain area of the limbic system, is particularly affected in AD in humans and

transgenic mice models. However, the effect that intracellular Aß may have on neuronal function has still not been examined. Therefore, in this study we analyzed the effects of intracellular Aß oligomers (iAßo) on acutely dissociated NAc neurons. To evaluate if iAßo could modulate components of the neurotransmission, we used a modified whole-cell patch clamp technique to dialyze Aßo intracellularly through the recording electrode. The effects of iAßo were study on the maximum evoked current (Imax) where under control conditions, the AMPA current was 149 ± 18 pA and decreased to 73 ± 15 pA after the application of iAßo 1 µM. Interestingly, GABA and GLY currents were not affected. Furthermore, iAßo was able to decrease accumbal neurons excitability, diminishing the number of action potential spikes and its amplitude. Overall, these findings showed that iAßo inhibited the amplitude of AMPA receptors in accumbal neurons and also decreased neuronal excitability. These effects support the notion that iAβo is able to impair neurotransmission in limbic areas.

93. FUNCTIONAL MODULATION AND MOLECULAR INTERACTION OF THE ALKALOID KOUMINE WITH GLYCINE RECEPTORS.

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Koumine is one of the main alkaloids of the Gelsemium genus plants. Behavioral studies have reported that the administration of koumine exerted analgesic and anxiolytic effects. The mechanisms underlying these beneficial effects are not well defined. However, behavioral studies have shown that the analgesic and anxiolytic effects of koumine are inhibited by strychnine, a selective antagonist of inhibitory glycine receptors (GlyRs), which are chloride-permeable pentameric ligand-gated ion channels expressed in the central nervous system. To date, whether koumine is able to modulate the function of GlyRs is unknown. Here, by using biochemical, electrophysiological and bioinformatics approaches, we studied the potential modulation of GlyRs by koumine. Our electrophysiological studies showed that koumine negatively modulates the GlyR function. For example, the acute application of 25 micromolar of koumine inhibited the glycine-activated current through recombinant alpha1-GlyRs and alpha3-GlyRs by ≈35%. Molecular docking studies based on the alpha3-GlyR crystal structure suggest that koumine interacts with the orthosteric pocket of the receptor, favoring a closed state of the ion channel. Ongoing biochemical studies will determine whether koumine directly interacts whit the extracellular domain of alpha3-GlyRs. Overall, these results demonstrate the actions of koumine on the GlyR function. These results, together with ongoing studies, may contribute to understand the mechanisms underlying the koumine-induced analgesia and anxiolysis.

94. PHARMACOLOGICAL INHIBITION “IN VIVO” OF OVARIAN ACETILCOLESTERASE REVERTS POLIQUISTIC OVARY PHENOTYPE IN RAT.

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Ovarian function is subject to endocrine and nerve regulation. An increase in sympathetic tone by cold stress (CS) induces a phenotype like polycystic ovarian condition (PCO). On the other hand, a local cholinergic system has been described in rat ovary, in which we find acetylcholine (ACh), the muscarinic receptor M1, and the enzyme acetylcholinesterase (AChE). Chronic treatment with the AChE inhibitor Huperzine A (Hup-A) has been reported to increase the fertility on the rat. In this context, the purpose of this study is to determine whether the long-term changes induced by CS on ovarian function can be reversed by increasing ACh chronically by administering Hup- A. In this study, Sprague-Dawley rats were subjected to CS subsequently hemiovarioectomized and implanted with a miniosmotic pump with Huperzine A (10 µM) or subjected to the procedure but without the implantation of miniosmotic pump (Sham). 28 days after the procedure the ovary and the serum were collected to measure steroid hormones Testosterone (T), Progesterone (P4) and Estradiol (E2) by enzyme immunoassay and the follicular development by morphometry. A second group of rats were used to measure the fertility after mate with males of proven fertility. The results show that CS generates a polycystic phenotype with cysts, hyperandrogenism and low fertility.

The administration of Hup-A reverses the alterations in follicular development and hyperandrogenism produced by CS but not increase the fertility. The pharmacological potential of these findings gives to the cholinergic local system relevance in the treatment of PCO.

95. DEVELOPMENT OF ACPV-56 LOADED MICROPARTICLES, FOR THE TREATMENT OF RHEUMATOID ARTHRITIS. PRELIMINARY STUDY.

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Rheumatoid arthritis (RA) is a chronic disease whose worldwide incidence is increasing. Currently, there are non-pharmacological and pharmacological approaches for the therapeutic management of RA. From the latter, the drug ACPV-56 has demonstrated anti-arthritical activity due to its ability to inhibit the proliferation of activated lymphocytes, which results in a marked anti-inflammatory effect. Clinical studies have proven that upon oral administration of therapeutic doses, ACPV-56 causes gastrointestinal adverse effects that negatively influence the compliance to chronic treatments. A strategy to avoid the adverse gastrointestinal effects of ACPV-56 and increase the dosing time intervals is by incorporating the drug into a controlled release system administered by intramuscular injection. As none of the available formulations containing ACPV-56 is intended for parenteral administration, the objective of this research is to develop a drug delivery system based on biodegradable microparticles (MPs) encapsulating ACPV-56. The MPs, elaborated by spray drying of a mixture of anionic polysaccharides, cationic and phospholipids, were characterized in terms of its in vitro release kinetics, employing conditions that emulate the physiological environment. The formulation parameters were optimized in order to obtain MPs suitable for injection. The obtained micro particles were spherical, with a medium diameter close 20 μm, relatively mono-disperse and with a minor tendency to aggregation. The incorporation of ACPV-56 did not affect the physicochemical properties of the developed MPs. Preliminary findings of the release kinetics showed that the encapsulation of ACPV-56 within MPs delays its release at least 10 times when compared to the free drug. As a conclusion, the developed microparticles represent a promising alternative for treatment of RA.

96. DEVELOPMENT OF CHEMICALLY CROSS-LINKED HYDROGELS WITH POTENTIAL BIOMEDICAL APPLICATIONS.

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Hydrogels based on natural origin polymers have shown promising results as scaffolds for cell encapsulation and drug delivery in tissue engineering. In this work, biopolymeric hydrogels, from natural and biocompatible polymers, were produced by two methods, chemical cross-linking with glutaraldehyde and freeze-thawing. The hydrogels were characterized using scanning electron microscopy (SEM) and Fourier-transform infrared spectroscopy (FTIR). The SEM images showed that the structure and morphology of the hydrogels produced by chemical cross-linking differed from those produced by freeze-thawing, while FTIR analysis also revealed different chemical composition between them. Their potential biomedical application was also assessed. First, their biocompatibility with HEP-2 cell line was tested using an MTT assay. The results showed that the chemically cross-linked hydrogels did not affect the cell viability compared to the freeze-thawing-produced hydrogels. We further tested the potential of chemically cross-linked hydrogels to retain and release bioactive compounds in the cells by loading the hydrogels with BSA protein conjugated with FITC. Using a fluorescence microscope, we observed that the HEP-2 cells were stained green, indicating a successful release of the conjugate. These data suggest that our chemically cross-linked hydrogels have the potential to be used for drug delivery in tissue engineering applications.

97. ANTIDEPRESSIVE LIKE EFFECT INDUCED BY THE ACUTE ADMINISTRATION OF IBOGAINE AND NORIBOGAINE IN RATS AND ITS POSSIBLE MECHANISM OF ACTION.

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Previous human subjective data and animal studies demonstrated that the psychedelic alkaloid ibogaine, and its metabolite noribogaine, have potent antiaddictive effects. The biological mechanism through both compounds elicit this beneficial effect remains still unclear. Among several molecular targets, ibogaine and noribogaine inhibit the serotonin transporter (SERT). This action and a longterm increase of brain-derived neurotrophic factor RNAm levels in the rat prefrontal cortex that we found in our previous study after the acute ibogaine i.p. administration, lead us to hypothesize that the anti-addictive property of ibogaine and noribogaine could be related to a potent putative antidepressant-like effect. Consistent with this possibility we characterized the behavioral effects (dose and time-dependence) induced by the acute ibogaine (20 and 40 mg/kg) and noribogaine (20 and 40 mg/kg) administration in rats using the forced swimming test (FST). Fluoxetine (40 mg/kg/i.p.) a standard antidepressant drug, was used as a control. We found that ibogaine and noribogaine induced a dose- and timedependent antidepressive-like effect. To know if the antidepressive-like effect induced by ibogaine was due to an effect per se or by the presence of its metabolite (noribogaine) we intravenously injected animals with ibogaine. Ibogaine 1 and 5 mg/kg after an i.v. injection on animal behavior immediately evaluated in the FST. Under these conditions, ibogaine did not generate an antidepressant effect. Ibogaine seems to depend on noribogaine content to induce the beneficial effect. All the behavioral responses were consistent with the pharmacokinetic data. Interestingly, noribogaine 40 mg/kg elicited an antidepressant-like effect per se with a higher potency than fluoxetine. Our data support the possibility that this potent antidepressive-like action could collaborate, at least in part, to explain the ibogaine´s previous anti-addictive effects.

98. MODULATION OF ANTIOXIDANT ACTIVITY IN DERIVATIVES OF AMINOETHYL PHENANTRENE AND HALO- APORPHINES WITH ANTINEOPLASTIC ACTIVITY.

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In the search for new molecules with antineoplastic and antioxidant properties derived from the natural product boldine (1), it was proposed to increase the molecular lipophilicity of the precursor molecule with the expectation of improving the above mentioned bioactivities. Using (1) as starting matherial was prepared: secoboldine (N, N-(methyl) (ethyl) phenanthrene; seco-boldine (2)) and a series of halo derivatives (halo = Cl or Br) (3-6), which were characterized by NMR-1H and -13C and the measurement of their melting point. Boldine (1) has the lowest dissociation energy in the phenolic group of C-9 with respect to C2-OH which is consistent with the highest acidity recorded for first phenolic group. The insertion of one halogen atom (3,4) maintains the energy value to break the O-H bond, but in compounds that bearing two halogen atom (5,6) the dissociation energy markedly increases while antioxidant capacity decrease in these compounds. This experimental evidence is correlated with the "local softness" (LS) exhibited by boldine and halogenated compounds. In conclusion, the antioxidant activity in these compounds can be modulated by the insertion of halogen atoms in the appropriate positions.

99. GALLIC AND GENTISIC ACID DERIVATES INDUCE AUTOPHAGY IN MURINE AND HUMAN COLORECTAL CANCER CELL LINES.

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Colorectal cancer is the third most common neoplasm in the world. The standard treatment consists mainly in surgery and the use of three first-line chemotherapies, 5-fluorouracil, oxaliplatin and irinotecan. The high drug resistance, several side effects and high costs of the treatments, give an opportunity to search for new molecules with new pharmacological targets. In the recent years, cancer cell mitochondria have become in an interesting pharmacological target due to their high mitochondrial-transmembrane potential that allows accumulated cationic probes conjugated with cytotoxic pharmacophore. In our laboratory, the gallic and gentisic acid derivatives as conjugated with lipophilic cationic triphenylphosphonium through an aliphatic chain of ten carbons have been tested in breast and colorectal cancer cells. These compounds triggered a series of events that leads cell apoptosis. The objective of this work is described how the decrease of ATP levels induces the activation of AMPK, which promotes death by autophagy in colorectal cancer lines. Through western blot and luminescence assay, we observed that in human and murine colorectal cell lines, the derivatives induce the reduction in cellular ATP levels followed by the activation of AMPK and LC3B, leading to cell death by autophagy. In conclusion, our compounds may be inducing apoptosis by triggering mitochondrial unbalance, energy stress and autophagy.

100. ANTIPROTOZOAL ACTIVITY OF CHICORY (CICHORIUM INTYBUS) AGAINST TRYPANOSOMA CRUZI.

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Chagas disease is an endemic parasitosis in Latin America. However, its drug treatment frequently induces adverse effects. Thus, it is urgent to develop new therapies. Chicory is a bioactive plant with antiparasitic activity related with its content of sesquiterpene lactones (SL). Most antiparasitic studies of chicory have been focused on nematodes, but poorly explored against parasitic protozoa. The aim of this study was to evaluate the antiprotozoal effects of SL-rich chicory extracts against Trypanosoma cruzi, the etiological agent of Chagas disease. SL of chicory leaves and roots were extracted from 5 chicory cultivars (Spadona, Goldine, Larigot, Measeto and Benulite) using methanol/water and purified by solid-phase extraction. The extracts were dissolved in DMSO. SL profiles of the extracts were characterised by UHPLC-MS metabolomics. The cytotoxicity of extracts was tested on T. cruzi trypomastigotes (Y strain) and mammalian Vero cells. Trypomastigotes were incubated for 24 h with serial dilutions of extracts (100-6.3 µg/mL), and benznidazole was used as positive control. Vero cells were exposed to extracts for 24 h at 100 and 50 µg/mL to evaluate cytotoxicity. Cell viability was evaluated by resazurin reduction test. Chemical profiling showed that chicory extracts have distinct content of SL among cultivars and between plant parts. All the extracts had dose-dependent effect against isolated trypomastigotes. However, Spadona leaf extract was the only with no toxicity against Vero cells at 100 µg/mL, suggesting a selective trypanocidal activity. Taken together, these results revealed that chicory Spadona leaf warrants deeper exploration regarding the relationship between its chemical profile and antiprotozoal activity. Consequently, these results encourage further investigation of chicory as a source of SL with antiparasitic therapeutic potential.

101. AUTISTIC AUTOANTIBODIES ABSORBED FROM BREAST MILK GENERATES COGNITIVE IMPAIRMENT IN BREEDING FEMALE BUT NOT MALE RATS.

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Autism spectrum disorders (ASD) involve a range of complex neurodevelopmental disorders, characterized by social impairments, communication difficulties,

and restricted, repetitive and stereotyped patterns of behavior. ASD exerts a significant physiological, emotional and financial burden on the families of the individual and society as a whole. Recently, beside the knowledge about genetic factors involved in this pathology, there is new evidence related to immunological causes of ASD. Therefore, it is of outmost importance to elucidate the molecular and physiological mechanisms of ASD pathology. Data from us and others have shown that normal young rat hippocampal slices incubated with purified IgA autoantibodies from ASD patients and breeding rats from pregnant mothers injected with the same antibodies, impairs LTP as well as disrupts learning and memory. Taking this into account, we hypothesized that ASD autoantibodies are absorbed from breast milk and generates autoimmune-related cognitive impairment characteristic of ASD pathology. To achieve this aim, we used a rat model where mothers were injected with ASD autoantibodies during breast milk period and the breeding was tested after that period using learning and memory test together with electrophysiological and immunohistochemical studies. We found that both LTP and learning and memory were significantly impaired in female but not male breeding rats and this alteration are correlated with the presence of ASD autoantibodies in hippocampus and Cortex. These results demonstrate that ASD autoantibodies are incorporated from breeding milk, cross both intestinal and blood-brain barrier and impairs learning and memory in a sex-preference fashion. They also give us new knowledge about possible causes of autism and opening a new line in pharmacological therapies.

102. MDMA (3,4-METHYLENEDI OXYMETHAMPHETAMINE) AND HELPING BEHAVIOR: PRELIMINARY CHARACTERIZATION IN SPRAGUE-DAWLEY RATS.

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MDMA (3,4-methylenedioxymethampheta mine, "ecstasy") is a psychotropic drug that induces an "open mind" state in healthy humans characterized by heightened self-acceptance, openness to communication and a fear threshold decrease known as the entactogenic syndrome. Disregarding its therapeutic potential, direct evaluation of MDMA-like effects in animal models remained limited to the pro-social paradigm, a model that recreates different stereotyped rodent behaviors. In contrast to these classical pharmacological criteria, helping behavior is a complex type of pro-social paradigm that has been recently described in rodents. It stands out because of its pertinence to develop a more sophisticated pharmacological model to study human-like behaviors, as it may occur in rats as a result of the interaction between psychomotor capabilities and the amount of stress experienced by the animal, even in the absence of reward. Despite of its relevance, the behavioral characterization of the effects of MDMA in this model remains unexplored. In the present work, a preliminary characterization of the effects of MDMA on helping behavior in male rat pairs (helper rat + victim rat; with/without previous individual housing) after 12 days-administration/ training cycles at two different dose levels (5 mg/kg; 10 mg/kg i.p.) has been attempted using a slightly modified water-trap model developed ad hoc. The results obtained indicated that MDMA might not enhance helping behavior compared to controls when the acting roles of each pair member has not been interchanged. In contrast, current data seems to be in agreement with the notion that helping assistance may rather depend on if the rat pair met each other previously or not and/or the experience of being trapped in the water trap, at least at the dose ranges evaluated.

103. EFFECT OF A LACTOBACILLUS ADMINISTRATION ON ANXIETY-LIKE BEHAVIORS IN ADULT RATS.

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There is increasing evidence that gut microbes affect central nervous system

(CNS) function. For instance, there are some Lactobacillus strains with proven anxiolytic and antidepressant like effects in rodents. However, there is also evidence that other Lactobacillus have anxiogenic effects in rats. In this regard, our previous findings show that 2 week administration of the potential probiotic bacteria Lactobacillus casei L-54-2-33 to healthy pre-pubescent Sprague-Dawley male rats, increased anxiety like behaviors, while lowering hippocampal expression of 5-HT1A receptor. Therefore, we asked if this anxiogenic-like effect was due to the rat's young age (post-natal day [PND] 35). To test this, we administered 104 CFU/ml of L. casei L-54-2-33 in the drinking water of male Sprague-Dawley rats from PND65 till PND76, and compared their anxiety-like behaviors with age and sex matched control rats fed with vehicle (sucralose and MRS broth) in the drinking water for the same amount of time. Anxiety-like behaviors were then evaluated using the open field (OF) test and elevated plus maze (EPM). Rats fed with the bacteria spent significantly less time in the central zone of the OF in comparison to controls, while there were no differences between bacteria fed and controls rats in the EPM. These results match with our previous findings in younger male rats, suggesting that the anxiogenic effects of L. casei L 54 2 33 are strain specific, and that these effect do not depend on the age of the animal. Together our findings suggest that bacteria known to promote changes in the CNS, might exert its strain-specific effects regardless of the age of the host, which is a novel feature in probiotic (or in this case psychobiotic) interventions

104. ANXIOLYTIC EFFECTS OF A CHILEAN EXTRACT OF HUMULUS LUPULUS.

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Introduction: Humulus lupulus is broadly cultivated in the world both for beer manufacture, but also for its medicinal properties, for the treatment of excitability and restlessness. There are important regional differences in metabolome composition of the plants that influence its response. The present study investigated whether a Chilean Humulus lupulus extract, previously selected by demonstrate antioxidant properties (enzymatic and in vitro assays), can elicit effects on the central nervous system, using various experimental models in rats.
Methods: a) Treatments: Two different doses (low or high) of Humulus lupulus extract from a regional variety, or Medi-Drop-sucralose used as vehicle (control group), were orally administered for 42 days to adults male Sprague-Dawley rats. Extracts were administrated one hour before the behavioral tests performed in this study. b) Open field was used for the evaluation of locomotor activity. Anxiety was evaluated by elevated plus-maze test (EPM). Results: No significant differences were observed on the locomotor behavior of rats, following the oral administration of two doses of extract or control, measured by total distance travelled and average speed, on the open field apparatus. But rats treated with low dose of extract significantly increased (16,75 +5,1 sec) the time spent on the central zone of the open field, compared to control (4,7+ 1,7 sec); this parameter is correlated with statistically difference observed with open arm spent time on the EPM between control and medium dose of extract. Conclusion: These results show that the low dose of this Chilean lupulus extract, could exert and anxiolytic effect.

105. PRODUCTION OF RECOMBINANT HUMAN INTERLEUKIN-4 EXPRESSED IN ESCHERICHIA COLI AS INCLUSION BODIES.

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Interleukin-4 (IL-4) is a potent lymphoid cell growth factor that stimulates the growth and survivability of certain B cells and T cells. It exhibits anti-inflammatory responses and participates in immune processes by providing protection from intracellular pathogens. IL-4 also plays an important role in T helper cells differentiation. Additionally, it can suppress pro-inflammatory cytokines. In this work, a His-tagged recombinant human IL-4 was overexpressed in Escherichia coli under the control of a T7 promoter. The resulting

inclusion bodies were separated from cellular debris by centrifugation and solubilized by 8M urea. The denatured IL-4 was refolded in a single chromatographic step by gradual removal of denaturant agent. This protocol yielded 4.5 mg of IL-4 from 40g of biomass. The refolded protein was highly pure and subsequent biological activity assay that was measured in the human erythroleukemia cell line TF-1 suggested that IL-4 had similar activity profile to the commercial produced protein. The results of this study suggest that on-column refolding represent a convenient and low-cost process for the refolding of IL-4 and may be a promising candidate for development as commercial reactive for cancer research.

106. A-KINASE ANCHORING PROTEIN AKAP79 INTERACTS WITH THE INTRACELLULAR DOMAIN OF THE GLYCINE RECEPTORS ALPHA SUBUNITS.

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Glycinergic inhibition is critical for breathing control, muscle tone regulation and nociception. Studies showed that PKA phosphorylation in the residue S346 located in the intracellular domain (ICD) of the glycine receptor (GlyRs) containing the alpha 3 subunit. This posttranslational modification produces inhibition of glycinergic function in the spinal cord, which was related to the generation of inflammatory chronic pain. Noteworthy, the molecular mechanisms associated with the inhibition of a3GlyR by phosphorylation are not yet elucidated. In this context, the interaction of the A-kinase anchoring protein (AKAP79) to partners involved in nociceptive pathways has been recently reported. Specifically, it has been observed that disruption of the interaction between AKAP79 and TRPV1 decreases sensitization in nociceptive neurons. Furthermore, the direct interaction between AKAP79 and the beta3 and beta2 subunits of the GABAAR promote the PKA-mediated phosphorylation of serine residues located in the ICD of those subunits. Nonetheless, whether AKAP79 is able to bind GlyRs and modulates its function is still unknown. Here, by using immunocytochemical and GST pull-down assays, we reported a direct association between the a1, a2 and the a3 subunits of the GlyRs with AKAP79. Confocal imaging showed that AKAP79 specifically co-localized with all the GlyRs subunits. In addition, in pull-down studies we observed that a GST-ICDa3GlyR fusion protein are able to bind AKAP79.Thus, our experimental data contributes to the characterization of a new intracellular partner of the GlyRs. This open new avenues in the searching of new therapeutic targets for the inflammatory chronic pain treatments.

107. PERINATAL ASPHYXIA INDUCES LONG-TERM DEMYELINATION, OLIGODENDROCYTES DAMAGE AND NEUROINFLAMMATION IN RAT BRAIN: PREVENTION BY NEONATAL MESENCHYMAL STEM CELLS TREATMENT.

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The effect of perinatal asphyxia (PA) was evaluated on myelination, oligodendrocytes, neuroinflammation and cell death in rat telencephalon and hippocampus from postnatal (P)1 up to 14 days, a period characterized by a spur of neuronal networking, finding a sustained injury that may have profound adverse effects on neuronal development. The study evaluated whether that injury could be prevented by mesenchymal stem cells (MSCs) treatment. PA was induced by immersing foetus-containing uterine horns into a water bath at 37°C for 21 min. Asphyxia-exposed (AS) and sibling caesarean-delivered (CS) foetuses were resuscitated and nurtured by surrogate dams. Animals were euthanized at P1, 7 or 14, dissecting samples from telencephalon and hippocampus to be assayed for (i) myelin (MBP and transcriptional factors involved in repairing demyelination, Olig-1and 2; immunofluorescence, RT-PCR); (ii) oligodendrocyte density (immunofluorescence); (iii) neuroinflammation (RT-PCR, ELISA,

immunofluorescence), and (iv) cell death (TUNEL). Two hours after delivery, AS and CS neonates were injected with either 5 µl of vehicle or 5x104 MSCs into the left lateral ventricle. It was found that PA produced: (i) a decrease of MBP density and oligodendrocyte/mm3 at P7 in telencephalon, but not in hippocampus; (ii) an increase of Olig-1, in telencephalon at P7; (iii) an increase of IL-6 mRNA levels in telencephalon at P7, and of IL-1β mRNA in hippocampus at P14; (v) an increase of cell death, including oligodendrocyte at P7 in telencephalon; (vi) MSCs treatment prevented the effect of PA on demyelination, oligodendrocyte density, neuroinflammation and cell death. It is proposed that PA induces regionally and developmental-dependent changes in brain regions, and MSCs treatment can prevent the changes induced by PA on myelination, oligodendrocyte density, neuroinflammation and cells death.

108. MHC-CLASS I POLYPEPTIDE-RELATED SEQUENCE A (MICA) AS AN IMMUNOTHERAPEUTIC TARGET IN CANCER.

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MICA is a ligand to NKG2D, an activation receptor that triggers natural killer (NK) cells effector functions for early tumor elimination; however, the normal function of MICA/NKG2D axis is compromised in cancer, including gastric adenocarcinoma (GA). Several mechanisms have been proposed to explain this response, including the presence of released MICA, either as soluble proteins or in exovesicles, which may favor down-modulation of NKG2D in cytolytic cells, resulting in desensitization of NK cells as the tumors progress. MICA is a highly polymorphic molecule that codifies allelic variants, which have been described to affect NKG2D binding avidity and cell cytotoxicity, while some MICA-STR variants located in the transmembrane domain promote NKG2D internalization. MICA-STR A5.1 variant acquires a GPI-anchor which is recruited in exosomes. The aim in this work was to evaluate the MICA expression in gastric adenocarcinoma and their relationship with the allelic variants and effect on the regulation of NKG2D receptor. We study the MICA expression and release in samples of tumor tissue by flow cytometry and ELISA assay. We isolated DNA genomic to determinate the MICA allele by sequence based-typing PCR using specific primers. Also, we evaluate the expression of NKG2D in tumor- infiltrating NK cells by flow cytometry. Our results indicated that the expression of NKG2D on NK cells was inversely proportional to the levels of MICA on tumor cells, and that not all patients showed detectable levels of soluble MICA (sMICA) in their serum and, while the diminished expression of NKG2D on cytolytic cells did not correlate with the concentration of sMICA in the serum of GA patients, this could be due to the presence of MICA in exovesicles as the MICA-STR A5.1 variant. In conclusion, we propose that MICA is an immunotherapeutic target in gastric adenocarcinoma and the MICA allelic variants should be considered in the therapeutic strategies.

109. INTERNALIZATION MECHANISM OF FOLATE-MODIFIED PAMAM DENDRIMERS IS MEDIATED BY MORE THAN ONE ENDOCYTOSIS PATHWAY.

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Nowadays, central nervous system (CNS) diseases affect 1.5 billion people worldwide and there is a continuous development of new therapies. However, in many cases efficiency of therapies is low because of biological barriers and deficient biodistribution of drugs. New advances in the nanomedicine have allowed the creation of nanotransporter systems. Among them, polyamidoamine (PAMAM) dendrimers have demonstrated a great potential in drug delivery to CNS. PAMAM dendrimers are polymeric structures composed by an ethylenediamine core that branches creating layers, called generations, which end in primary amines protonated at physiological pH and can be modified with other terminal groups, such as folate. Considering the current difficulty of delivering drugs to the CNS, we examined the internalization mechanism of folate-conjugated PAMAM dendrimers mediated by folate receptor α (FRα), a membrane

protein overexpressed in choroid plexus that once it binds to folate is internalized by the caveolae endocytosis pathway, and is postulated as a target tissue for drug delivery to CNS. In this study, we selected the HeLa cell line for internalization experiments, based on confocal and western-blot results. One unmodified (G4) and two folate-modified (PFO25 and PFO50) fourth generation PAMAM dendrimers were used. Confocal images showed that PFO50 was not able to entry HeLa cells, unlike PFO25 and G4, which were visualized after one hour incubation. Quantification of Mander's coefficients indicated only a slight increase of colocalization of PFO25 with FRα than unmodified G4, which suggests that the internalization pathway of folate-modified dendrimers is possibly mediated by more than one endocytosis mechanism.

110. EFFECT AQUEOU EXTRACT OBTAINED FROM LEAVES OF U. MOLINAE AND THEIR RESPECTIVE PRODUCTS OF GASTOINTESTINAL DIGESTION ON THE VIABILITY OF COLON CANCER CELLS.

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Colorectal cancer is the third most common diagnosis in men (10%) and second in women (9.4%). The use of chemotherapy to fight this disease leads side effects, this the main reason for investigations of possible antiproliferativity of different natural sources. In that regard, active compounds U. molinae and their products the gastrointestinal metabolized could act as prophylactic and complementary because effcts anticancer has been reported.The aims is To asses the effect aqueou extract obtained from leaves of U. molinae and their respective products of in vitro gastrointestinal digestion on the viability of colon cancer cells. Ugni molinae leaves were used to prepare an aqueous extract, with this a gastrointestinal disgestion was performed, obtaining also a final residue. These samples were evaluated in different viability tests, such as trypan blue exclusion, metabolic activity (MTT) and cytotoxicity (LDH), on colorectal cancer cells (CaCo-2) and healthy cells (HEK) for a period of 24 hours. When treating the cells, it is observed that the count of viable CaCo-2 cells decreases as the concentration increases. In the case of HEK cells no changes in the count are observed. MTT assay only with the gastrointestinal digestion samples observated an effect of inhibition of the metabolic activity in the case of caco-2 cells, in hek cells there is no significant effect. Cytotoxicity assays using LDH do not show significant changes in the activity of the enzyme lactate dehydrogenase in any case. Finally, it is concluded that the samples have positive effect on viable cell count and MTT assay, because damage colorectal cancer cells (caco-2) but not healthy cells (HEK), while very mild cytotoxicity was observed at through the LDH assay

111. ASSESSMENT OF DIFFERENT PHARMACOLOGICAL ACTIVITIES OF PEUMUS BOLDUS EXTRACTS USING CHEMICAL SUBTRACTION STRATEGY.

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Peumus boldus Mol., (Monimiaceae) is a Chilean medicinal three used for gastrointestinal and liver diseases. Phytochemical profiling of this plant is based on its aporphine alkaloids, phenolic compounds and essential oil. However, in herbal infusions some authors thought that flavonoids are responsible for its antioxidant and chemopreventive effects rather than alkaloids and essential oil. The objective of this study was to evaluate different knock-out extracts prepared by chemical subtraction oriented to selectively remove alkaloids and essential oils from crude extracts. These extracts were obtained by means of conventional centrifugal partition chromatography (CPC) and pH-zone-refining CPC. DPPH bleaching test, cytotoxicity in AGS cells, DNA damage in monocytes (Comet assay) and inhibition of Acetylcholinesterase were determined for all extracts. Solutions of the different lyophilized extracts were prepared at different concentrations (1-1000 ug/mL). The results of DPPH assay indicated an IC50 of 63.05, 109 and 43.73 ug/ml for

total extracts, alkaloids and polyphenols respectively, the fraction containing the polyphenols having greater antioxidant capacity. In turn, cytotoxicity tests showed that polyphenols at concentrations lower than 1000 ug/mL protected AGS cells. On the contrary, alkaloid fraction reduced cell viability from 400 ug/mL whereas fraction containing essential oil displayed higher toxicity from 125 ug/mL. Only the fraction with alkaloids displayed an expected acetylcholinesterase inhibition.

112. PARTICIPATION AND ROLE OF CONNEXINS IN THE RELEASE OF GLUTAMATE AND ATP IN THE NEURULATION PROCESS IN XENOPUS LAEVIS.

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Neurulation is an important process in the formation and development of CNS. This event correspond to the first step of neural embryonic development (stg 12,5–20 in Xenopus laevis) and implicates different cellular process like, migration and proliferation. Alterations in the signaling of this period could result in neural tube defects (NTDs). Several studies has demonstrated the participation of connexins (Cxs) as hemichannels in cellular communication through the release of ATP and glutamate, regulating cellular migration and stabilizing synaptic transmission. In this investigation, we identified the presence of several Cxs during neurulation. To evaluate its relative expression, we identified their RNA transcripts in different stages of Xenopus laevis neural embryonic development such as: stg 10; stg 12,5; stg 14 and stg 20. Our results revealed the presence of transcripts of Cxs 43, 45, 46, 32 and 26 in different stages of Xenopus laevis development. The more important proteins correspond to Cx 46 (GJA3) which has 6 fold expression vs Cx 45 (GJA7) and Cx 43 (GJA1). In turn, Cx 32 (GJB1) have a significant presence of 3 fold vs Cx 26 (GJB2), the second more abundant, during neurulation. Later, we decided to evaluate the functionality of these Cxs as hemichannels through pharmacological blockage assays, using inhibitors such as carbenoxolone (cbx) and enoxolone (enx). We found values of IC50 of ~30 μM and ~20 μM for cbx and enx respectively in their capacity to induce neural tube defects. In addition, in silico studies using molecular docking techniques, we determine the possible site of cbx and enx association in the protein, located in the extracellular domain (E2). Taken together these results, we suggest that Cx 46 and Cx 32 will participates as hemichannel in neural tube closure and their blockade results in NTDs.

113. INHIBITION OF ENDOPLASMIC RETICULUM EXIT RESCUES A NIEMANN PICK TYPE C DISEASE MODEL.

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Currently, more than 70 lysosomal diseases have been identified, accumulating substrates in lysosomes and late endosomes. Within this group we find the Niemann-Pick type C (NPC) disease, that generates aberrant accumulations of cholesterol and other lipids within cells, resulting in early neuronal death. NP-C1, the most common protein showing disease-causing mutations, codes for a transmembrane protein NPC1, present in lysosomes and late endosomes membranes. NP-C2, is caused by a mutation in the NPC2 gene that encodes the NPC2 protein, which is soluble and present in the same organelles. The disease produced by loss of function of NP proteins generates hepatomegaly and splenomegaly. Based on preliminary laboratory data, we hypothesize that proteins related to the organization of the endoplasmic reticulum (ER) are necessary to maintain the disease phenotype. In order to identify other proteins involved, we studied Tango1, a transmembrane ER protein that organizes vesicle cargo. Its loss of function produces disorganization and stress of the ER. It was analyzed in a model of Drosophila melanogaster where NP-C1 is replicated with a knock-down of dnpc1a gene, NPC1 ortholog, through RNAi. Using this system, we determined that tango1 loss of function reverts NP-C1 phenotype, improving Drosophila larval development progression. Also, the effect of the inhibition of ER secretion was analyzed using Fli-06, a compound that inhibits exportation. We tested a pharmacological model that phenocopies NP-C1, completely reverting NPC phenotype. The study corroborates that the organization of the secretory pathway is determinant to maintain the phenotype of

this disease and that by itself an alteration in it results in a phenotype equivalent to the deficiency of NPC1.

114. ADDITIVE EFFECT OF MODAFINIL AND CAFFEINE ON THE LOCOMOTIVE ACTIVITY OF ADULT RATS.

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Currently, many people are subject to a high work and academic load that leads them to consume psychotropic substances to be able to carry out these highly demanding activities. In Chile, it has been observed that some of these substances commonly used are modafinil and caffeine to promote wakefulness and concentration. Concomitant use of modafinil and caffeine could trigger anxious symptoms and psychomotor agitation in humans. Modafinil has different action mechanisms, being the blocking of dopamine transporter (DAT) the most known and relevant. On the other hand, caffeine is an adenosine receptor antagonist and inhibitor of phosphodiesterase. Therefore, the concomitant use of modafinil and caffeine could promote a higher effect on neural activity compare to the use of caffeine or modafinil alone. The objective of this work was to measure the additive effects of the administration of modafinil and caffeine on the horizontal and vertical locomotor activity. To assess the traveled distance and number of bipedestation, we used rats treated with caffeine (20 mg/kg, i.p.), modafinil (80 mg/kg, i.p.) and caffeine plus modafinil. Our preliminary results show that rats treated with caffeine plus modafinil produce an increase on locomotor activity (horizontal and vertical) compared to the administration of caffeine or modafinil alone. The effect induced by caffeine plus modafinil was additive. To correlate these behavioral effects with an increase in dopaminergic activity in the mesolimbic and nigrostriatal pathways, we will measure the dopamine release in striatum and nucleus accumbens using in vivo brain microdialysis and fast scan cyclic voltammetry

115. CLINICAL CHARACTERISTICS OF NEUROLOGICAL PATIENTS INFECTED WITH HTLV-1 AND DETERMINATION OF THE LOCATION OF TAX VIRAL PROTEIN.

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Tropical Spastic Paraparesis neuropathogenesis (abbreviated HAM / TSP, "HTLV-1-associated myelopathy / tropical spastic paraparesis"), endemic in Chile, shows by anatomo-pathological studies spinal cord injuries due to axonal loss and demyelination of cortical spinal beams, visualized by NMR. 70% of patients start their disease with paretospastic gait. Since 2009 the screening of HTLV-1 in blood banks. The prevalence in donors studied in the ISP in PBMCs ("Peripheral Blood Mononuclear Cells") containing T-CD4+ lymphocytes, the main viral reservoir, showed real-time prevalence of 1.2 healthy / 1000 individuals. Neuropathogenesis is attributed to the viral protein Tax because the virus does not infect neurons and 40% of patients with paraparesis are seronegative for the virus, but express a tax gene. In cerebrospinal fluid (CSF) of patients we detect Tax (by ELISA) and in isolated cells (by immunofluorescence) and in plasma (by "Western Blot"). Tax secreted from patient PBMCs agrees with the extracellular role that we propose, because we know that it interacts with semaphorin-4D that triggers the disassembly of microtubules and actin fibers through Plexin-B1.

116. POLYMERIZATION ACTIVITY AND CYTOTOXICITY OF MOLECULES WITH AFFINITY FOR LAU/PLA BINDING SITE OF TUBULIN AS NOVEL STABILIZING AGENTS.

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The importance of microtubules in cellular division set these proteins as pharmacological targets for antimitotic agents, known as tubulin binding agents (TBA), which can promote stabilization or destabilization of tubulin polymerization. The occurrence of adverse drug reaction associated to several of these agents drives the need for the development of new TBAs with a safer pharmacological profile. In this regard, a combination of computational virtual screening, molecular dynamics and binding free energy estimations was performed by our group, based on the stabilizing LAU/PLA binding site of tubulin. A set of 7 candidates were proposed as potential stabilizing agents with affinity for the site. In this work, we confirm the polymerization capacity for these 7 candidates in vitro at concentrations of 50 and 100 µM. Also, we observed an additive effect of the compounds when co-treating with Taxol, confirming a non-competitive binding with taxane-site binders. Finally, viability assays in a cancer cell line were developed showing a cytotoxic effect of molecules at 100 µM. These results set a starting point of further studies for the characterization of the novel agents that will open possibilities for the rational screening of new tubulin stabilizing agents.

117. RELEASE AND UPTAKE KINETICS OF DOPAMINE ARE PRESERVED IN STRIATUM OF ADULT FEMALE RATS EXPOSED DURING FIRST HOURS OF POSTNATAL LIFE TO ESTRADIOL VALERATE.

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Sex hormones play an important role in regulating reproductive and non-reproductive tissues, such as the brain. In the nervous system, sex hormones are important in its development and neural plasticity, however changes in the sex hormones milieu during fetal or neonatal stages affect brain function and generate persistent changes until adulthood. During last 7 years our lab has been interested in study how neonatal exposure to sex hormones such as estradiol valerate (EV) affect the functionality of midbrain dopaminergic neurons of adult male and female rats. The aim of this work was to evaluate the release and uptake kinetics of striatal dopamine (DA) induced by methylphenidate (MPH: 5.0 mg/kg i.p.) of adult female rats exposed during the first hours of postnatal life to estradiol valerate (EV: 0.1 mg/50 uL of sesame oil s.c.) or vehicle (50 uL of sesame oil s.c.). Our results did not show significant differences in the voltammetry parameters such as peak amplitude, area and tau (time constant of decay). Despite these results, we cannot rule out changes in the voltammetry parameters in nucleus accumbens, a key nucleus of the reward circuit, where we have previously observed a reduction in DAT expression of animals programmed with sex hormones.

118. CYTOTOXIC EFFECTS CAUSED BY DELOCALIZED LIPOPHILIC CATIONS DERIVED FROM POLYHYDROXY-BENZOIC ACIDS IN COMBINATION WITH DOXYCYCLINE ON LUNG CANCER CELLS.

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Lung cancer has the highest mortality between all neoplasms, being the second leading cause of death in Chile. These cancers are classified as small cell carcinoma or non-small cell carcinoma, being Smoking the main risk factor. Conventional treatments are radiotherapy, chemotherapy and surgery; however, 5-year survival rates remain extremely poor, due to the development of resistance and eventual relapse. The Cancer Stem Cells hypothesis suggests that they are responsible for tumor initiation and growth and are resistant to conventional treatments. Therefore, it is necessary to develop new therapies that allow us to effectively eliminate resistant tumor cells (TC). Mitochondria may be considered as a therapeutic target in the treatment of cancer, because it exhibits a greater transmembrane potential in TC, being susceptible to being the target of positively charged molecules. The delocalized lipophilic cations of triphenylphosphonium (TPP+) are molecules synthesized from gallic acid, mono

and polybenzoates decyl esters. Therefore, we evaluated 4 decyl polyhydroxybenzoate compounds linked to TPP+ as potential cytotoxic agents in two lung cancer cell lines (NCI-H727 and NCI-H1299) and in pulmonary fibroblasts (PH) as control. Doxycycline is an antibiotic of the tetracycline group; recently its has been studied this antineoplastic use producing the inhibition of mitochondrial biogenesis in TC. Cell viability assay was performed with the compounds at 24, 48 and 72 hours in normoxia and hypoxia with 5% oxygen to determine IC50, to subsequently perform a combination test of compounds with doxycycline. The results showed that the TCT analyzed are sensitive to the cytotoxic action of all compounds and this effect is increased as time goes by. In addition, there are no significant differences in IC50 between hypoxia and normoxia cultures.

119. TARGETING MITOCHONDRIAL METABOLISM IN NEURODEGENERATIVE DISEASES THROUGH NCLX BLOCKADE.

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The loss of mitochondrial function is part of the almost all neurodegenerative diseases. Therefore, there's a reduction in ATP synthesis ending up in a dysfunction of neuronal dynamics. At least three proteins from the Krebs' circle are calcium sensitive. In our laboratory, we're focus in the design and synthesis of CGP37157 derivatives, reference blocker drug of the mitochondrial sodium/calcium exchanger (NCLX), which also has neuroprotective properties. Our aim is the discovering of new pharmacological tools able to handle calcium flux between mitochondria and cytosol, lowering the calcium overload descried in the cytosol. Putting together these two ideas, we wondered if the partial blockade of mitochondrial calcium efflux could improve not only mitochondrial metabolism but also calcium handling. The CGP57137 derivative selected was ITH12575, a benzothiazepine with a different aromatic substitution. First, the calcium movements were studied by the fluorescent dye Fluo4, in the human neuroblastoma SH-SY5Y cell line and in embryonic cortical neuros of rat primary culture. In order to confirm ITH12575 selectivity for NCLX, the exchanger was silenced by a siRNA and neuroprotective assays were evaluated. Finally, mitochondrial stress assays were performed using the seahorse method, in presence/absence of both a toxic stimulus (high potassium concentration) and the compound. Data from ATP synthesis, mitochondrial respiration and respiratory maximal capacity were obtained. The results show that, by the regulation of mitochondrial calcium, the mitochondrial metabolism is partially recovered thanks to ITH12575.

120. FOXO1 MEDIATES HIGH GLUCOSE-CARDIAC FIBROBLASTS DIFFERENTIATION

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Normally cardiac fibroblasts (CF) maintain the homeostasis of the extracellular matrix (ECM) in the heart, whereas in pathological conditions such as diabetes, become more active promoting cardiac fibrosis. High glucose (HG) induces CF differentiation, where TGF-beta1 has a crucial role. TGF-beta1 requires FoxO1 to induce CF differentiation, whereas FoxO1 is deregulated in diabetes, resulting in its hyperactivation, oxidative stress and cell differentiation. Therefore, in this work we wanted to determine the role of FoxO1 in CF differentiation promoted by high glucose. CF obtained from adult Sprague-Dawley rats were incubated in HG, as a in vitro model of hyperglycemia. CTGF and alpha-SMA expression was determined by RT-PCR, whereas CTGF and alpha-SMA protein were evaluated by westernblot (WB). The activation of FoxO1 was analyzed evaluating its phosphorylated forms, its nuclear localization and the expression of FoxO1 specific genes targets (p21cip and p15ink) by RT-PCR. The oxidative stress was evaluated analyzing the expression of the FoxO3a, catalase and SOD2 proteins by WB, and ROS production by colorimetry. The role of FoxO1 was demonstrated using AS1842856 (FoxO1 inhibitor) and FoxO1 silencing using siRNA. HG increased the protein and mRNA of CTGF and alpha-SMA (CF differentiation marker), whereas HG decreased of AKT activation, decreased phospho-s256-FoxO1 level, increased FoxO1 nuclear localization and increased FoxO1 genes target expression

(FoxO1 activation marker). Likewise, HG decreased FoxO3a, catalase and SOD2 protein, and increased ROS production. In addition CF differentiation induced by HG was completely abolished by FoxO1 inhibition using AS1845628 and FoxO1 silencing. Collectively these data suggest that FoxO1 is necessary to CF differentiation induced by high glucose and suggest that FoxO1 would be a pharmacological target for new treatments against diabetic cardiomyopathy.

121. PHARMACOLOGICAL COMPARISON BETWEEN PREFRONTAL CORTEX AND NUCLEUS ACCUMBENS NEUROTRANSMITTER LEVELS AFTER BASOLATERAL AMYGDALA STIMULATION.

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Glutamatergic neurons of the basolateral amygdala (BLA) innervate both, the nucleus accumbens (Nac) and prefrontal cortex (PFC) (McDonald, 1991). The relation between BLA and Nac has been implicated on the control of motivated behavior (Stuber et. al., 2011). Furthemore, the triade between BLA, Nac and PFC has also been shown to be critical in the regulation of goal-directed behavior by an inhibitory control of PFC on Nac dopamine release during amygdala activation (Jackson et. al. 2018). BLA has been implicated in fear, anxiety and stress (Simon et. al., 2014; Janak and Tye, 2015). The stress response is centered in the corticotrophin releasing factor (CRF) system (Bale and Vale, 2004). There are two major receptors for CRF in the brain, type-1 and type-2 CRF receptors (CRF-R1 and CRF-R2). Several studies have shown a significant role of CRF-R1 in the stress response; however, the role of PFC and Nac CRF-R2 in the stress response is poorly understood. We studied the role of CRF-R2 in PFC and Nac neurotransmitter levels after BLA stimulation by double in vivo microdialysis in PFC or Nac of anesthetized adult rats. Local infusion of antisauvagine 30 (CRF-R2 antagonist) in the PFC or Nac significantly increased PFC and Nac dopamine and glutamate extracellular levels induced by BLA stimulation. Our results suggest that there is an inhibitory tone mediated by CRF-R2 controlling dopamine and glutamate extracellular levels in PFC and Nac that dependon BLA stimulation.

122. ACTIVATION OF NMDA RECEPTORS DURING CHRONIC PAIN RECRUITS PROTEIN SRC KINASES TO OPEN PANNEXIN1 CHANNEL IN NEUROPATHIC RAT MODEL.

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In experimental pain models the upregulation of NMDA receptor (NMDAR) appears to be crucial in the enhanced responsiveness of nociceptive neurons of dorsal horn of the spinal cord for initiation and maintenance of pain. In the chronic pain model performed by our laboratory, it was shown that intrathecal injection i.t. of 10Panx (Inhibitor panx1 channel peptide) prevents the effect of hyperalgesia caused by i.t of NMDA in neuropathic rats suggesting an interaction between Panx1 channel and NMDAR. Our work is based on elucidating whether the regulation of the Panx1 channel by post-translational modifications is carried out by Src kinases in neuropathic rats model. In dye uptake experiments (used to assess Panx1 channel opening) of the spinal cord slices of neuropathic rats have demonstrated that pharmacological inhibition of PP2 (Src tyrosine kinase protein inhibitor), and 10Panx decreased dye uptake in neurons stimulated by NMDA. Likewise, rats treated with 10panx-NMDA or PP2-NMDA decreases the expression of phosphorylated Panx1 (pPanx1) and phosphorylated Src527 (pSrc527) by western Blotting. Algesymmetric test (Randall Selitto) results have shown that intrathecal administration of 10Panx (300 μM, 10μl i.t) -NMDA (0.6 mM, 10μl i.t) and PP2 (3.3 mM, 10μl i.t)-NMDA (0.6 mM, 10μl i.t) inhibit pain in neuropathic rats compared to control. We conclude that Panx1 or Src inhibition prevents nociceptive signaling induced by upregulation of NMDAR in neuropathic rats, suggesting that the pronociceptive effects of pharmacological activation of NMDAR induce the opening of the Panx1 channel probably mediated by Src kinase.

123. PARTICIPATION OF VGLUT AND GLUTAMATE SIGNALING DURING NEURULATION IN XENOPUS LAEVIS.

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Introduction: The development of the nervous system begins with the closure of the neural tube, one of the morphological changes present in the neurulation process. Several signaling pathways participate in the formation of the neural tube, such as FGF, Wnt, Chordin and glutamate, recently described. Failures in this process lead to the formation of neural tube defects (NTDs), the second more prevalent birth defect worldwide. Use of antiepileptic drugs during pregnancy had shown an increase in the incidence of NTDs by mechanism not fully understood yet. Here, we propose that glutamate, the principal excitatory neurotransmitter of the nervous system and specifically its vesicular transporter VGLUT1, participate in the normal closure of neural tube. Methods: X. laevis embryos were treated with Rose Bengal, a VGLUT1 antagonist, at early neurula stage (14 hpf, neural plate) until end of neurulation (21 hpf). Then, we perform a morphological evaluation of neural tube closure and immunofluorescence experiments. Additionally, after neurula treatments, behavior studies using Xenopus tadpoles (stg 45-49), were performed to evaluate epileptic sensibility by measure seizure latency onset using Pentylenetetrazol (PTZ). Results: We observe and incomplete neural tube closure in embryos treated with Rose Bengal in a dose-response manner, with an EC50 of 3.5±0.5µM. Furthermore, we observe a decrease of ~43% ($p<0.01$, ANOVA) in the seizure latency onset necessary for epileptogenic behavior induced by PTZ and a 5-fold increase (0.4±0.3 m vs 5±0.2m) in the distance traveled at 2 minutes after treatment ($p<0.05$, ANOVA). Conclusions: VGLUT1-mediated glutamate signaling participate in normal neural tube development. Partial blocking of this pathway at neurula modifies the epileptogenic-induce response in tadpoles, possibly by alter the normal establishment of the nervous system.

124. INDOMETHACIN IMPAIRS POLYAMINE METABOLISM IN LUNG CANCER CELLS: A KRAS MUTATION-ASSOCIATED FEATURE?

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Non-small cell lung cancer (NSCLC) is the most lethal and prevalent type of lung cancer. NSCLC patients carrying mutations in the Kirsten rat sarcoma viral oncogene homolog gene (KRAS) still lack targeted therapies. Also, the levels of polyamines (putrescine, spermidine, and spermine) are increased in cancer, playing a pivotal role in tumor proliferation. Indomethacin increases the levels of the polyamine-catabolic enzyme spermidine/spermine-N1-acetyltransferase (SSAT). Consequently, the aim of this study was to compare the effect of indomethacin in the polyamine metabolism of two NSCLC cell lines, with different KRAS mutation status. A549 and H1299 NSCLC cells (KRAS-mutated and wild-type, respectively) were exposed to indomethacin. Evaluations included SSAT expression and protein levels, and metabolic analysis of cells by CG-MS metabolomics. Moreover, the difference in polyamine synthesis enzymes among cell lines and the synergistic effect of indomethacin combined with inhibitors of these enzymes were investigated. Indomethacin increased the expression and levels of SSAT in both cell lines. In A549 cells, indomethacin significantly impairs polyamine metabolism. However, in H1299 cells, the impact of treatment on the polyamine pathway was non-significant. Evaluation of the levels of the polyamine synthesis enzymes showed that ornithine decarboxylase (ODC) is increased in A549 cells, whereas S-adenosylmethionine-decarboxylase (AMD1) and polyamine oxidase (PAOX), are increased in H1299 cells. Finally, indomethacin demonstrated a synergistic effect with the PAOX inhibitor MDL72527 in A549 cells, whereas in H1299 had a synergistic effect with the AMD1 inhibitors SAM486. Collectively, these results indicate that indomethacin alters polyamine metabolism in NSCLC cells and enhances the effect of polyamine synthesis inhibitors such as MDL72527 or SAM486. However, this effect varies depending on the basal metabolic fingerprint of each type of NSCLC cell. FONDECYT-1160807.

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