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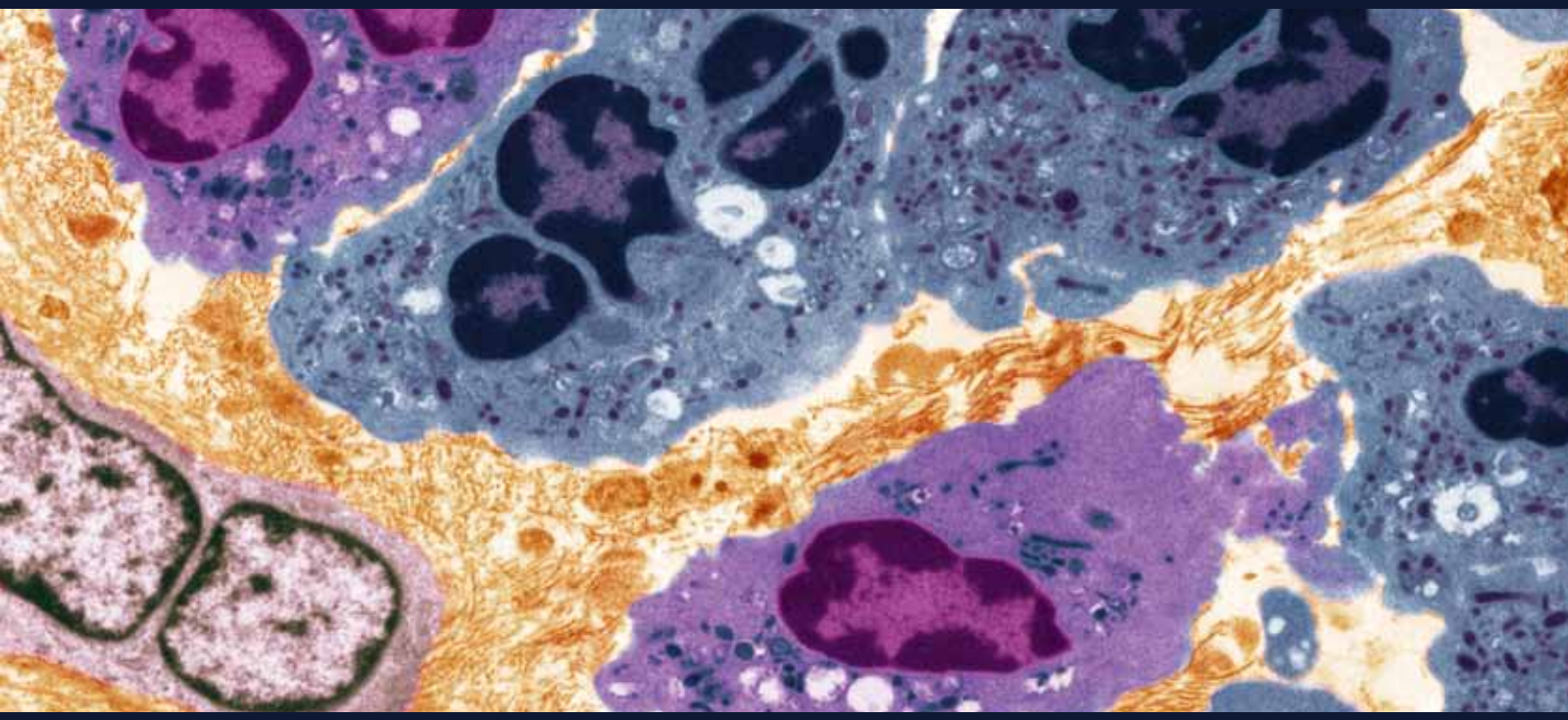
Ocular Inflammation and Infection

Guest Editors: Michelle Callegan, Meredith Gregory-Ksander, Mark Willcox, and Susan Lightman



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Editorial Board

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Editorial

Ocular Inflammation and Infection

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The eye is a unique and necessary organ that is constantly exposed to the environment. In an immune-privileged environment such as the eye, a delicate balance exists between immune responses that limit damage and those responses that can result in irreversible damage. Ocular inflammation and infection are therefore potentially blinding events. Efforts to analyze the delicate balance between helpful and harmful immune responses in the eye have involved a variety of animal studies and models which analyze not only the specific etiologic agents of infection and the contributions of their products in inflammation and vision loss but also the underlying host factors responsible for those responses. Other studies have probed the development of novel therapeutics based on endogenous host factors as well as pathogen-specific targets and have tested these with routinely used therapeutics in improved regimens in an effort to improve visual outcome. The ultimate goal is to provide patients with the appropriate treatment that rescues vision regardless of disease.

The scope of this special issue involves a wide variety of areas in ocular infection and inflammation highlighting recent developments in the epidemiology, pathogenesis, and treatment of various types of ocular infections and inflammation. The first paper of this special issue reviews the challenges of cataract surgery and visual rehabilitation in patients with uveitis. This discussion also includes management pearls in combating complications that arise in this particular group of patients. The second and third papers continue the theme of inflammation, analyzing chemotactic cytokine production in the cornea. The second paper reports a time course effect in the synthesis of IL-8 and MCP by stromal cells stimulated with LPS. The third paper uses an *ex vivo* model of herpes simplex virus 1 (HSV1) corneal infection to demonstrate that neutrophils are important in T-cell recruitment and control of HSV1 replication via synthesis of IP-10. The following three papers address epidemiological issues with studies on microbiological profiles and treatment outcomes of scleritis, chronic postoperative endophthalmitis, and fungal ocular infections. The key message of these studies is the importance of early identification and determination of drug susceptibility and proper therapeutic and/or surgical intervention in saving useful vision. The seventh and eight papers discuss the use of corticosteroid therapy in endophthalmitis and other types of ocular inflammation. The seventh paper reviews the visual outcome of cases of filtering bleb-associated endophthalmitis treated with or without intravitreal dexamethasone, while the eighth paper reviews the clinical use of loteprednol etabonate in a variety of ocular inflammatory conditions. The final paper of the special issue discusses hyperactivation of the renin-angiotensin system in inflammation and retinal neural dysfunction. The authors suggest that inhibition of this system may be a novel therapeutic approach to preventing or treating inflammation-based ocular diseases.

This special issue includes the following papers: "Cataract surgery in uveitis," "IL-8 and MCP gene expression and production by LPS-stimulated human corneal stromal cells," "Resident corneal cells communicate

with neutrophils leading to the production of IP-10 during the primary inflammatory response to HSV-1 infection," "Clinico-microbiological profile and treatment outcome of infectious scleritis: experience from a tertiary eye care center of India," "Chronic postoperative endophthalmitis: a review of clinical characteristics, microbiology, treatment strategies, and outcomes," "Support of the laboratory in the diagnosis of fungal ocular infections," "Intravitreal dexamethasone in the management of delayed-onset bleb-associated endophthalmitis," "Advances in corticosteroid therapy for ocular inflammation: loteprednol etabonate," and "Renin-angiotensin system hyperactivation can induce inflammation and retinal neural dysfunction."

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Review Article

Chronic Postoperative Endophthalmitis: A Review of Clinical Characteristics, Microbiology, Treatment Strategies, and Outcomes

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Chronic postoperative endophthalmitis (CPE) is a delayed infectious intraocular inflammation process that occurs more than six weeks after ocular surgery and frequently masquerades as autoimmune uveitis. These cases are at risk of delayed diagnosis and erroneous long-term treatment with corticosteroids. This paper aims to review the epidemiology, microbiology, clinical characteristics, diagnosis, management strategies, and outcome of chronic postoperative endophthalmitis. The incidence of CPE is still uncommon, and multiple pathogens have been reported with varying frequencies. Review of the literature reveals that CPE cases have a high incidence of visual impairment and recurrence rate might be decreased with aggressive surgical approach.

1. Introduction and Definitions

Endophthalmitis is an uncommon but sight-threatening intraocular inflammation that may be due to a noninfectious process or may be caused by an infectious organism. It is a term used to describe intraocular inflammation that involves the vitreous cavity and the anterior chamber of the eye and can involve other adjacent ocular tissues such as the choroid or retina, sclera or cornea [1]. In infectious endophthalmitis, the organism might reach the eye from other infected sites in the body through hematologic seeding and in these cases it is labeled endogenous endophthalmitis. More commonly, the organism is exogenous and gains access to the intraocular environment [2]. According to the Endophthalmitis Vitrectomy Study, postoperative endophthalmitis is divided generally into two types: acute and chronic. Acute postoperative endophthalmitis is defined as infections within 6 weeks of surgery; on the other hand, chronic postoperative endophthalmitis is defined as infections after 6 weeks of surgery [3].

The term chronic postoperative endophthalmitis (CPE) was first coined in 1986 in a case series of 15 patients by Meisler et al. [4]. The inflammation is usually indolent and may persist for months. It is often misdiagnosed as noninfectious iritis where it improves initially with topical corticosteroid therapy while flaring whenever corticosteroids are tapered or stopped [5]. This is in contrast to acute postoperative endophthalmitis, which presents as a single episode of severe inflammation with an acute onset that usually follows surgery by a few days but can be delayed more than a week in some cases. As such, acute and chronic postoperative endophthalmitis are two clearly different clinical entities [2, 4, 5].

2. Epidemiology

Postoperative endophthalmitis is an uncommon complication of any ocular surgery. The reported incidence of postoperative endophthalmitis ranges from 0.01% to 0.367%, with incidence varying among different surgical procedures and across studies and different countries [1, 6–11]. Most of postoperative endophthalmitis studies were conducted on cases after cataract surgery, being the most commonly performed surgery in ophthalmology [11]. In a large meta-analysis, 3 140 650 cataract extraction cases were reviewed for the incidence of endophthalmitis after cataract surgery worldwide in the period between 1964 and 2003 [12].

The analysis showed an increase in the incidence of postsurgical endophthalmitis from 0.087% in the 1990s to 0.265% in the 2000s, and this was attributed to the change in surgical technique towards clear corneal sutureless wounds that allow exogenous organisms easy access to the intraocular space.

Furthermore, postoperative endophthalmitis has been reported after pars plana vitrectomy, penetrating keratoplasty, trabeculectomy, and glaucoma drainage device surgeries. Endophthalmitis also has been reported following exter nal ocular surgeries such as scleral buckle, pterygium excision, and strabismus surgeries [11]. The highest endophthalmitis rate was found in surgical procedures associated with cataract extraction reaching 0.367%; on the other hand, pars plana vitrectomy was found to have the lowest incidence rate with only 0.04% especially after using microincision tech nique [11, 13].

The data regarding the incidence of chronic postoperative endophthalmitis are still lacking. But this form of post operative endophthalmitis appears less common than the acute variety [14]. Some reports estimated the ratio of acute to chronic postoperative endophthalmitis to be between 5 : 1 and 2 : 1, indicating that the incidence rate of chronic postoperative endophthalmitis can be 5 per 10000 [15]. In one single-center study, the reported rate of chronic onset endophthalmitis following cataract surgery was 0.017% [16].

3. Etiology, Microbiology, and Pathogenesis

The organisms causing chronic postoperative endophthalmitis tend to be different from the acute form pathogens [14]. They are usually indolent bacteria or fungus with low virulence. CPE was originally considered to be a reaction to the remaining native lens tissue and was consequently called toxic lens syndrome or phacoanaphylactic endophthalmitis [17]. However, studies of removed lens capsules revealed small gram-positive rods, consistent with *Propionibacterium acnes*, adherent to the capsular remnants [17].

A variety of organisms have been implicated in chronic postoperative endophthalmitis (Table 1), with *Propionibacterium* species accounting for the majority of cases (41 to 63%) followed by coagulase-negative *Staphylococcus* and fungus [5, 16, 18].

Propionibacterium acnes, formerly known as *Corynebacterium parvum*, is a variably staining, gram-positive, pleomorphic, and anaerobic bacillus. As its name suggests, *P. acnes* is associated with chronic skin infections and with the contamination of a variety of prosthetic devices [19, 20]. Despite being a potent stimulant of the immune system, *P. acnes* is largely resistant to the killing mechanisms of monocytes and neutrophils, which enables it to persist intra-cellularly after phagocytosis [21].

Reviewing the largest three case series of CPE revealed that 48% of the cases are caused by *P. acnes*, followed by fungal organisms in 21% of the cases and gram-positive species in 16% of the cases (Table 2).

Some case reports have also isolated *Actinomyces*, *Nocardia*, *Achromobacter*, *Cephalosporium*, *Acremonium*, *Paecilomyces*, *Ochrobactrum* and *Aspergillus* species as causes of CPE [22, 29–31]. In some of these organisms such as *Staphylococcus epidermidis,* and *Propionibacterium acnes*, the clinical course of the disease may be affected by factors such as host characteristics or inoculum size [2, 5].

Table 1: Infectious pathogens isolated in chronic postoperative endophthalmitis [5, 16, 22–27].

Bacterial pathogens	
*Propionibacterium acn*es	*Ochrobactrum anthropi*
Staphylococcus species	*Hafnia alvei*
Corynebacterium	*Sphingomona spaucimobilis*
Nocardia	*Mycobacterium chelonae*
Cephalosporium and Acremonium	*Pseudomonas stutzeri*
Paecilomyces	*Achromobacter*
Fungul pathogens	
Aspergillus species	*Fonsecaea pedrosoi*
Candida species	*Paecilomyces* species
Curvularia lunata	*Acremonium strictum*

Table 2: Percentage of organisms reported in different case series of chronic postoperative endophthalmitis [5, 18, 28].

Pathogens	Shirodkar	Al-Mezaine	Fox	Percentage
Propionibacterium acne	11	7	12	48.3%
Gram-positive species	3	3	4	16%
Gram-negative species	3	1	0	6.4%
Mycobacteria	2	0	0	3.2%
Fungal species	7	3	3	21.3%
Mixed	0	3	0	4.8%

Routes of bacterial entry are believed to include intraoperative irrigation fluids, surgical instruments, and inadvertently placing the intra-ocular lens on external ocular surfaces [32, 33]. The anterior chamber possesses an efficient mechanism of clearing small bacterial loads, so the currently unexplainable failure of this mechanism may be one of a multitude of unknown factors in postoperative bacterial endophthalmitis [32, 34]. Known risk factors include vitreous communication (e.g., through a posterior capsular tear or YAG capsulotomy), certain IOL prosthetics, and diabetes [35–38].

Fungal endophthalmitis is uncommon in the postoperative setting, with most of the cases being attributable to *Candida* species [5]. As such, most fungal endophthalmitis cases are the result of infection by filamentous fungi, and a minority is the result of molds [39]. Fungi possess resistant cell walls that enable them to flourish in the eye indefinitely shielded from immune attack and antibiotic therapy making the management of these cases particularly challenging [40, 41].

4. Symptoms and Clinical Finding

The clinical picture of CPE is that of a recurrent and often low-grade uveitis occurring months or even years after the

inciting surgical event. Uveitis typically starts two to three months postoperatively and involves the anterior chamber initially with progression to the vitreous as the disease advances. Pain or discomfort may or may not be present in CPE, while decreased vision is found in nearly all patients. Inflammation is usually steroid responsive initially but recurs after medication tapering, while it paradoxically worsens with steroids in the case of some fungal infections [42]. The clinical course in CPE is similar to that of phaeoantigenic uveitis and has been suggested to be a result of an immune reaction to the presence of both residual lens material and bacteria [43, 44]. A slit lamp eye examination will reveal white blood cells in the anterior chamber. The uveitis may be granulomatous with large precipitates on the cornea or intraocular lens and often without a frank hypopyon, but a microhypopyon may be visible by gonioscopy. A white intracapsular plaque representing retained lens particles and sequestered organisms is highly suspicious of an infectious process [14]. The plaque is commonly observed especially in association with *Propionibacterium* species and less frequently with other bacterial or fungal infections [16, 29, 45, 46]. Vitreous activity is usually mild but can be dense and diffuse particularly with *Staphylococcus epidermidis* [5]. CPE of fungal etiology is usually characterized by "pearls-on-a-string" or "fluff balls" near the capsular remnant and also with stringy white infiltrates although both are not pathognomonic [5, 14].

5. Diagnostic Approach

The diagnosis of CPE is challenging given the difficulties faced in isolating the causative organism. It is based on clinical suspicion supported by cultures of the aqueous or posterior lens capsule or vitreous biopsy [47]. When CPE is suspected, aqueous and/or vitreous samples should be obtained for analysis. The sampling could be performed using needle aspiration of 0.01 mL of the aqueous fluid or 0.02 mL of the vitreous. In case the vitreous needle aspiration was not successful (dry tap), mechanical biopsy of the vitreous through a pars plana vitrectomy could be performed. The obtained sample should be analyzed with gram stain, culture, and identification of antimicrobial sensitivities [14]. The appropriate anaerobic medium should be used when necessary and Giemsa and fungal cultures should be obtained in case a fungus is suspected. The highest diagnostic yield is achieved by sampling the white plaque in the posterior lens capsule if present, utilizing a special culture medium, as well as prolonging the culture time to several weeks to cover the slow-growing organisms implicated in CPE [14, 48]. In culture negative cases, the additional use of polymerase chain reaction was reported to aid in the identification of the organism [49]. The utilization of a universal bacterial primer could be of help in this setting.

CPE differential diagnosis spectrum includes noninfectious causes such as lens-induced uveitis secondary to retained cortical material, IOL-induced uveitis secondary to implant malposition causing iris chafing and chronic inflammation, and sympathetic ophthalmia or other causes of uveitis unrelated to surgery [50, 51].

6. Treatment Strategies and Outcomes

The indolent nature of the organisms and their sequestration within the capsule protected from host defenses along with their different virulence factors make it hard to define a treatment protocol for CPE or extrapolate the guidelines set for acute postoperative endophthalmitis [14].

Different modalities of treatment approaches have been reported, and they range from (1) intraocular antibiotics injection (IOAB) only to, (2) pars plana vitrectomy (PPV) with IOAB to, (3) PPV with IOAB and partial capsulectomy to, (4) PPV with IOAB and total capsulectomy with IOL removal or exchange [5, 16, 18, 28]. In addition, some advocate waiting for culture, gram stain, and sensitivity data to allow for directed therapy in cases where the inflammation is not considered severe [2].

Two intraocular antibiotics injection approaches have been described either into the capsular bag or simultaneously into the aqueous and the vitreous [52, 53].

Some reports suggest tailoring treatment options to the severity of presenting signs and symptoms where mild cases are to be managed with intraocular cultures followed by intravitreal antibiotics while using repeated intraocular antibiotic and pars plana vitrectomy with partial capsulectomy in recurrent cases [42]. Another approach depends on the type of the isolated organism whereby *S. epidermidis* could be treated with intraocular antibiotic injections alone while *P. acnes* would require surgical intervention with pars plana vitrectomy, capsulectomy and possible removal or exchange of the IOL in addition to intraocular antibiotic injection [13, 20, 44]. This is based on the fact that multiple reports described high rate of recurrence when *P. acnes* CPE was treated with intravitreal antibiotics alone [20, 44].

Since at the time of the initial antibiotic injection the organism is usually unknown, the initial approach to consider in the empiric treatment of chronic postoperative endophthalmitis, when fungal infection is not suspected, is intravitreal vancomycin (1 mg/0.1 mL) owing to its broad coverage of gram-positive bacteria and methicillin-resistant *Staphylococci*. *P. acnes*, the most commonly described causative organism of CPE, is also sensitive to vancomycin but not to aminoglycosides [14, 15]. It has also been reported to have good susceptibility to carbapenems (meropenem and ertapenem) in vitro [54]. Accordingly, the treatment should be modified as sensitivity studies become available [15].On the other hand, the benefit of systemic and topical antibiotic use remains controversial in CPE [14].

A cross-sectional review of four of the biggest case series on delayed-onset endophthalmitis revealed differences in outcomes that can be attributed to causative organism, initial treatment modality, as well as the extent of intervention [5, 16, 18, 28]. A total of 98 patients with CPE were reported in these series. The overall visual outcome is calculated to be 20/40 or better in about 46% of the cases while 54% ended up with varying degrees of visual impairment, all irrespective of the stratifying factors mentioned above (Table 3).

All four case series indicate that an infection with *P. acnes* or gram-positive organisms was associated with a better visual outcome (better than 20/40 in 54.5% and 50% of

Table 3: Visual acuity outcomes reported in four major series of chronic postoperative endophthalmitis [5, 16, 18, 28].

VA outcome	Fox ($n = 19$)	Clark ($n = 36$)	Al-Mezaine ($n = 17$)	Shirodkar ($n = 26$)	Overall ($n = 98$)
≥20/40	9 (47.3%)	18 (50%)	5 (29.4%)	13 (50%)	45 (45.9%)
20/50 ≥ 20/400	6 (31.5%)	10 (28%)	4 (23.5%)	6 (23%)	26 (26.5%)
<20/400 ≥ 5/200	1 (5.2%)	2 (5%)	2 (11.7%)	2 (7.7%)	7 (7.1%)
<5/200-NLP	3 (15.8%)	6 (17%)	6 (35%)	5 (19.2%)	20 (20.4%)

Table 4: Visual acuity outcomes by causative organism in chronic post operative endophthalmitis [5, 16, 18, 28].

Organism	≥20/40	20/50 ≥ 20/400	<20/400 ≥ 5/200	<5/200-NLP
P. acnes ($n = 66$)	36/66 (54.5%)	20/66 (30%)	2 (3%)	8/66 (12%)
Gram positive ($n = 10$)	5/10 (50%)	2/10 (20%)	1/10 (10%)	1/10 (10%)
Fungal ($n = 13$)	5/13 (38.5%)	3/13 (23%)	2/13 (15%)	3/13 (23%)
*Others ($n = 9$)	1/9 (11%)	2/9 (22%)	0	6/9 (66.6%)

*Others: gram-negative, mycoplasma, and mixed organisms.

Table 5: Recurrence rate of chronic post operative endophthalmitis with different initial treatment modalities [5, 18, 28].

Initial treatment	Fox ($n = 19$)	Clark ($n = 36$)	Shirodkar ($n = 26$)	*Overall ($n = 62$)
IOAB only	4/5 (80%)	12/12 (100%)	2/3 (66%)	18/20 (90%)
PPV + IOAB	2/2 (100)	5/10 (50%)	8/10 (80%)	15/22 (68%)
PPV + PC+ IOAB	5/11 (45%)	2/14 (5.5%)	9/13 (69%)	16/38 (42%)
PPV + IOL exchange	0/1 (0%)	None	None	0/1 (0%)

*AL-Mezaine review series was not included since it did not mention the recurrence rate after initial treatment.

Table 6: Recurrence rate of chronic post operative endophthalmitis with different surgical interventions [5, 16, 18, 28].

Treatment modality*	Fox ($n = 19$)	Clark ($n = 36$)	Al-Mezaine ($n = 17$)	Shirodkar ($n = 26$)	Overall ($n = 98$)
PPV + IOAB	5/7 (71%)	5/10 (50%)	1/3 (33.3%)	10/12 (83%)	15/22 (68%)
PPV + PC + IOAB	1/9 (11%)	4/21 (19%)	0	9/13 (69%)	14/43 (32%)
PPV + TC + IOL exchange	0/4 (0%)	0/7 (0%)	0/4 (0%)	1/7 (14%)	1/22 (4.5%)
PPV + TC + no IOL	0	0/5 (0%)	0/1 (0%)	1/12 (8%)	1/18 (5.5%)

*At any time of treatment (initial, secondary, or tertiary intervention).

the overall cases, resp.) (Table 4). Fungal infection was associated with a more unfavorable prognosis where visual impairment was precipitated in more than 60%, and more than 20% had severe visual impairment (worse than 5/200). In the same pool of patients, the recurrence rate differed in relation to the initial treatment modality (Table 5). The highest recurrence was seen in cases where the initial treatment consisted of antibiotics alone (90%). Starting therapy with pars plana vitrectomy and antibiotics decreased the recurrence in all series, while adding posterior capsulectomy to pars plana vitrectomy and antibiotics as an initial management further decreased the recurrence rate to 42%. As a trend, all case series showed that recurrence rate decreased uniformly in correlation with a more aggressive management strategy (Table 6), whereby the overall calculated recurrence rate, when combined PPV, IOAB, total capsulectomy, and removal or exchange of the IOL was performed at any time during followup, decreased to as low as 5% compared to 68% recurrence rate when PPV was combined with IOAB alone (Table 6).

Chronic fungal postoperative endophthalmitis carries a poor prognosis and there is no standard management available for treating this very rare condition. Current approach includes pars plana vitrectomy, intravitreal amphotericin (5–10 mg/0.1 mL) or voriconazole, and a systemic antifungal drug [55–57]. The indolent course of the chronic fungal postoperative endophthalmitis might benefit from prolonged systemic treatment with an antifungal (6 weeks–6 months) [57]. Topical antifungal agents (natamycin 5%) are started when required, especially in cases of corneal involvement [57].

In conclusion, chronic postoperative endophthalmitis should always be in the differential of recurrent inflammation in a previously operated eye. A worsening course of inflammation despite treatment is particularly alarming. Effort should be directed towards finding a definitive diagnosis in this setting through obtaining intraocular samples for analysis early enough to institute aggressive treatment and avoid recurrence and poor outcome.

Conflict of Interests

The authors have no proprietary interests in the subject matter of the paper.

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Review Article

Advances in Corticosteroid Therapy for Ocular Inflammation: Loteprednol Etabonate

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Topical corticosteroids are effective in reducing anterior segment inflammation but are associated with adverse drug reactions (ADRs) including elevation of intraocular pressure (IOP) and cataract formation. Retrometabolic drug design has advanced the development of new corticosteroids with improved therapeutic indices. Engineered from prednisolone, loteprednol etabonate (LE) has a 17α-chloromethyl ester, in lieu of a ketone group, and a 17β-etabonate group. LE is highly lipophilic and binds with high affinity to the glucocorticoid receptor; any unbound LE is metabolized to inactive metabolites. LE has been studied in several anterior segment inflammatory conditions (giant papillary conjunctivitis, allergic conjunctivitis, anterior uveitis, and keratoconjunctivitis sicca), and in postoperative ocular inflammation and pain. Combined with tobramycin, it is effective in blepharokeratoconjunctivitis. Elevations in IOP are infrequent with LE, and the absence of a C-20 ketone precludes formation of Schiff base intermediates with lens proteins, a common first step implicated in cataract formation with ketone steroids.

1. Introduction

The eye is vulnerable to damage from relatively low levels of intraocular inflammation. The blood-aqueous and blood-retinal barriers usually limit penetration of protein and cells from the peripheral circulation, while regulatory molecules and cells in the eye actively suppress immunologic responses [1]. Nevertheless, ocular inflammatory conditions and surgical trauma induce changes in the blood-aqueous and blood-retinal barriers [1–3]. As a result, immune cells and mediators of inflammation enter the eye, resulting in the classical clinical signs and symptoms of ocular inflammation including redness, pain, swelling, and itching [4]. Ocular inflammation, if left untreated, may lead to temporary or permanent loss of vision [5].

Topical corticosteroids are useful for the management of anterior segment inflammation. Corticosteroids elicit numerous potent anti-inflammatory effects [6]. For instance, they suppress cellular infiltration, capillary dilation, the proliferation of fibroblasts, collagen deposition, and eventually scar formation; they stabilise intracellular and extracellular membranes; and they increase the synthesis of lipocortins that block phospholipase A_2 and inhibit histamine synthesis in the mast cells. Inhibition of phospholipase A_2 prevents the conversion of phospholipids to arachidonic acid, a critical step in the inflammatory cascade. Corticosteroids also increase the enzyme histaminase and modulate transcription factors present in mast cell nuclei.

Corticosteroids mediate their anti-inflammatory effects primarily through the modulation of the cytosolic glucocorticoid receptor (GR) at the genomic level [7, 8]. After corticosteroids bind to the GR in the cytoplasm, the activated corticosteroid-GR complex migrates to the nucleus, where it upregulates the expression of anti-inflammatory proteins and represses the expression of proinflammatory proteins. However, recent work suggests that the activated corticosteroid-GR complex also elicits nongenomic effects, particularly the inhibition of vasodilation, vascular permeability, and migration of leukocytes [7, 9]. In addition, corticosteroids mediate anti-inflammatory activity through membrane-bound GR-mediated nongenomic effects and through direct nonspecific interactions with cellular membranes [9, 10].

Because the GR is involved in a plethora of signalling pathways—in fact, more than 5000 genes are expressed or

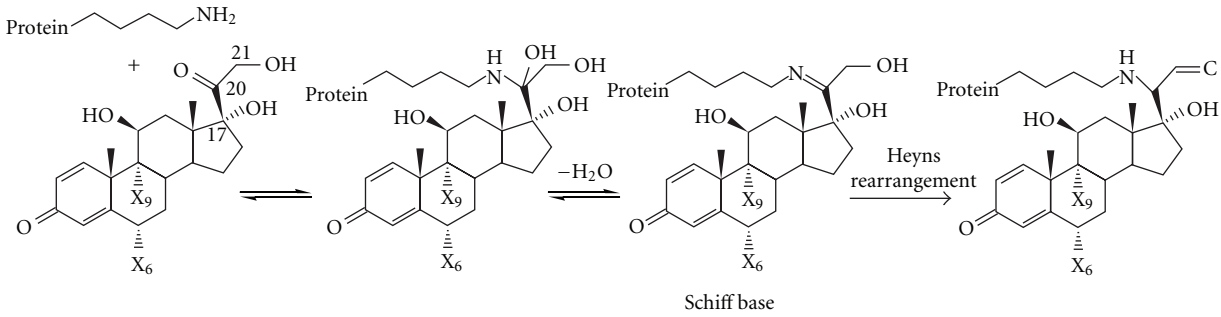


Figure 1: Mechanism of steroid-induced cataract formation adapted from [17].

suppressed following glucocorticoid exposure [11]—long-term use or high dosages of corticosteroids can result in adverse drug reactions (ADRs) such as increased IOP [12, 13]. Most studies implicate the involvement of trabecular meshwork (TM) cells and myocilin gene expression in the mechanism of corticosteroid-induced IOP increase. Steroids decrease the outflow of aqueous humor by inhibiting the degradation and/or enhancing the deposition of extracellular matrix material within the TM and/or cross-linking of actin fibres between TM cells [14]. Structural changes in the TM, in turn, result in corticosteroid-induced ocular hypertension, which can progress to secondary iatrogenic open-angle glaucoma [15]. Myocilin, initially referred to as TM-inducible glucocorticoid response or *TIGR* gene product, is a 55-kDa protein induced after exposure of TM cells to dexamethasone for 2-3 weeks, which is also closely associated with decreased aqueous humor outflow and steroid-induced IOP increase [16]. Different mutations within the myocilin gene lead to a variety of glaucoma phenotypes in both juvenile and adult-onset primary open-angle glaucoma, providing further evidence for its role in steroid-induced IOP [14].

Another ADR associated with corticosteroid use is the formation of cataract. However, the mechanism of steroid-induced cataract formation appears to be chemically based and not likely to be related to the downstream effects of GR activation. Currently, the most prominent hypothesis for cataract formation involves nonenzymatic formation of Schiff base intermediates between the steroid C-20 ketone group and nucleophilic groups such as ε-amino groups of lysine residues of lens proteins [17]. The formation of Schiff bases is followed by a Heyns rearrangement of the adjacent C-21 hydroxyl group, resulting in stable anime-substituted adducts (Figure 1) [17]. While this covalent binding mechanism could account for cataract formation with C-20 ketone-based corticosteroids, other mechanisms of steroid-induced cataract formation may exist. Interestingly, covalent adducts have been observed only in steroid-induced cataract, not in other cataracts.

Further research into the mechanisms of action of steroids—both for their anti-inflammatory effects and for ADRs—is underway. Herein, we review the design of new corticosteroids through retrometabolic design and review available data from preclinical and clinical studies of loteprednol etabonate (LE), the first retrometabolically designed topical steroid to reach marketing status. Studies confirming the premise of retrometabolic design are discussed.

2. Retrometabolic Drug Design

Only a small fraction of systemically administered drugs will distribute to the eye from the general circulation, and an even smaller fraction thereof will cross the blood-retinal barrier to reach the eye. Thus, topical administration of corticosteroids is the preferred route for anterior segment inflammatory conditions as it maximizes drug delivery to the anterior segment and minimizes systemic exposure. Topical administration also helps avoid systemic ADRs such as hypothalamic-pituitary-adrenal-(HPA-axis) suppression. Nevertheless, topical ophthalmic corticosteroids are associated with ADRs including elevations in IOP, cataract formation following extended use, delayed wound healing, and lower resistance to infection [1, 7]. As previously discussed, steroid ADRs appear to arise from the continued action of the corticosteroid-GR complex at the genomic level beyond the action required to elicit anti-inflammatory effects or, in the case of cataract formation, through formation of covalent bonds with lens protein.

In an effort to decrease ADRs, Bodor and colleagues developed the concept of retrometabolic drug design more than 30 years ago [18]. The underlying principle of retrometabolic drug design is to synthesize analogues of lead compounds or reference compounds, starting from a known inactive metabolite of that lead compound. The inactive metabolite is converted into an isosteric/isoelectronic analogue with structural modifications designed for rapid, predictable metabolism back to the original inactive metabolite after eliciting the desired therapeutic effect [19] (Figure 3). Although Bodor named such analogues "soft drugs," these analogues were predicted to have therapeutic potency similar, if not identical, to that of the lead compound, but, due to the structural modifications included by the design, any active drug remaining following attainment of therapeutic effect would be metabolically deactivated, thus minimizing any ADRs (hence, the "soft drug" terminology). However, the increase in therapeutic index could only be achieved if the drug was stable enough to reach its receptor to elicit the desired effect, while any free drug remaining thereafter would be metabolized to avoid ADRs. Metabolism that is too rapid would

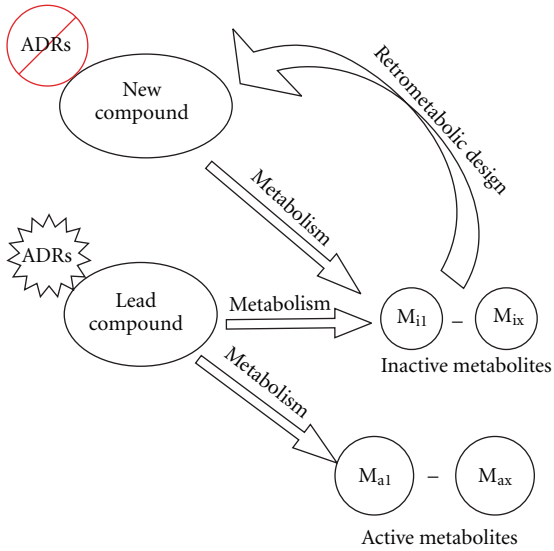
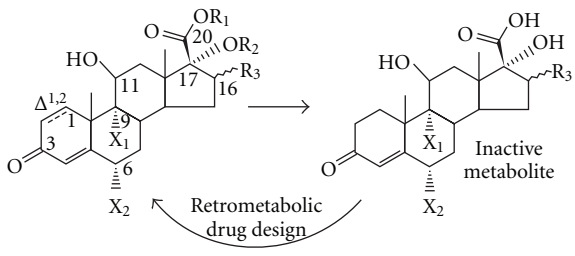


Figure 2: Concept of retrometabolic drug design in which a new lead compound is created based on an inactive metabolite of a previous lead compound.



R_1 = alkyl, haloalkyl, etc.
R_2 = alkyl, alkoxyalkyl, COOalkyl, etc.
R_3 = H,α- or β-CH_3
X_1, X_2 = H, F
$\Delta^{1,2}$ = double bond (present or absent)

Figure 3: Retrometabolic design of cortienic acid-based derivatives adapted from [52].

result in decreased efficacy as would poor bioavailability and/or poor GR-binding affinity. In other words, there had to be a balance between the solubility and lipophilicity of the drug, its tissue distribution and receptor binding, and subsequent rate of metabolic deactivation.

Over the years, Bodor and colleagues applied retrometabolic drug design to a variety of therapeutic agents including antimicrobials, β-blockers, analgesics, and acetylcholinesterase (ACE) inhibitors, with several retrometabolically designed compounds reaching marketing application. With respect to ocular corticosteroids, Bodor designed a number of analogues, starting with Δ1-cortienic acid, the primary metabolite of prednisolone, that lacks corticosteroid activity [19] (Figure 2). To obtain new lead compounds, the pharmacophore moieties of the 17α-hydroxyl and 17β-carboxy substituents of the lead compound had to be restored by suitable isosteric/isoelectronic substitution containing esters or other types of functions that restored the original corticosteroid's anti-inflammatory potency while incorporating hydrolytic features to ensure metabolism. Other structural considerations included the presence/absence of double bond at the Δ1 position, fluorination at 6α carbon (X_2) and/or 9α carbon (X_1), and methylation at 16α or 16β carbons (R_3). Over a hundred possible drugs were synthesized and tested in preclinical anti-inflammatory models, and structure/activity studies concluded that the best substitutions for maximal activity included a haloester at the 17β position and a carbonate or ether at the 17α position. 17α esters were also considered but were quickly abandoned due to their potential to form mixed anhydrides with the haloesters, and subsequent potential for lens protein binding. Thus, in addition to the C-20 ketone moiety of prednisolone being replaced to avoid the possibility of formation of Schiff base intermediates, other chemical features associated with potential cataractogenesis were also eliminated by design.

3. Loteprednol Etabonate

3.1. Preclinical Studies. The most promising drug candidate among cortienic acid-based derivatives synthesized by Bodor and colleagues was loteprednol etabonate (LE; chloromethyl 17α-ethoxycarbonyloxy-11β-hydroxy-3-oxoandrosta-1,4-diene, 17β-carboxylate) [20]. LE is the 17β-chloromethyl ester of Δ1-cortienic acid with a 17α-etabonate moiety and was predicted to undergo rapid deesterification to an inactive carboxylic acid metabolite after exerting its effect, thereby minimizing the likelihood of toxicity.

Selection of LE for further development was based on a number of criteria. LE is highly lipophilic—its lipophilicity is 10 times greater than that of dexamethasone, a characteristic that may increase its efficacy by enhancing penetration through biological membranes [21]. Further, competitive binding studies with rat lung type II GRs demonstrated that the binding affinity of LE was 4.3 times that of dexamethasone [22]. A vasoconstriction test in humans used to assess bioavailability showed that LE produced a blanching response similar to that of betamethasone 17α-valerate, thereby confirming good penetration properties and strong potency [12]. But more importantly, initial studies by Bodor showed that the therapeutic index of LE was more than 20-fold better than that of other corticosteroids including hydrocortisone 17α-butyrate, betamethasone 17α-valerate, and clobetasone 17α-proprionate based on the cotton pellet granuloma test and thymolysis potency [9].

Studies in animals confirmed that LE is indeed predictably metabolized by local esterases into its inactive metabolite, Δ1-cortienic acid. Druzgala et al. [23] studied the ocular absorption and distribution of ^{14}C-labelled LE 0.5% in the eyes of rabbits. The highest concentrations of LE were found in the cornea, followed by the iris/ciliary body and aqueous humor. The cornea also showed the highest ratio of metabolite to LE, indicating that the cornea was the primary site of metabolism, while aqueous humor concentrations of LE were approximately 100-fold lower. This finding suggested that LE may exert a decreased IOP effect relative to other corticosteroids, as high levels of steroids in the aqueous humor are thought to contribute to decrease outflow

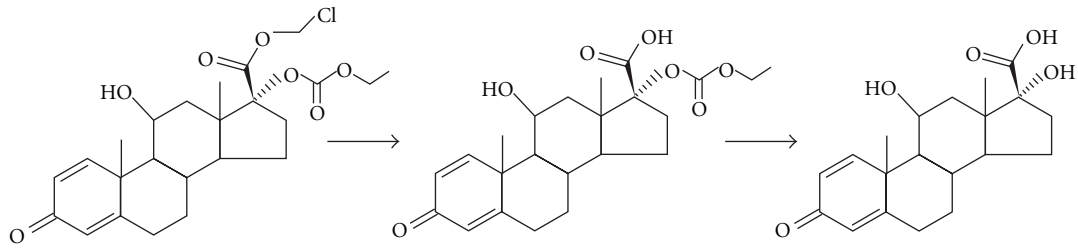


Figure 4: Metabolism of loteprednol etabonate.

through the TM. LE was found to have a terminal half-life ($t_{1/2}$) of 2.8 hours in dogs following intravenous administration [24]. Further, when absorbed systemically, LE was found to be metabolized to Δ1-cortienic acid etabonate and then to Δ1-cortienic acid (Figure 4) and eliminated rapidly through the bile and urine [20, 25].

More importantly, a comparison of the IOP-elevating activity of LE with that of dexamethasone in rabbits confirmed a lack of IOP effect with LE [26]. LE and dexamethasone, both at 0.1% concentrations, and vehicle were instilled topically 8 times per day for 2 days to normotensive rabbits in a 3-way crossover design. Treatment with dexamethasone produced an increase in IOP of ∼4 mm Hg after only 8 instillations, while there was no significant difference in IOP in animals treated with LE versus those treated with vehicle.

More recently, Glogowski and Proksch [27] studied the ocular pharmacokinetics of LE in rabbits with corneal inflammation. Consistent with results obtained by Druzgala et al., high concentrations were found in the cornea and conjunctiva, while low levels were found in the aqueous humor. The C_{max} and $AUC_{(0–24\,h)}$ were, respectively, 3.62 (5.47) μg/mL and 6.10 μg · h/g in the conjunctiva, 1.40 (1.45) μg/mL and 3.30 μg · h/g in the cornea, and 0.0293 (0.00805) μg/mL and 0.0838 μg · h/g in the aqueous humor. These results confirm good corneal and conjunctival penetration of LE into the anterior segment, while hydrolysis limits significant aqueous humor accumulation. In addition, Samudre et al. studied the efficacy of LE compared to other corticosteroids in a model of ocular inflammation—lipopolysaccharide-induced uveitis in rabbits [28]. It was found that LE 0.5% induced greater GR migration to the nucleus as compared to prednisolone acetate 1% and fluorometholone 0.1%. This effect correlated with the disappearance of inflammatory cells from the corneal stroma and restoration of corneal endothelium.

Numerous additional preclinical studies have been conducted to date on LE in addition to these presented here. Taken together, they demonstrated that LE achieves the required balance between the solubility/lipophilicity, ocular tissue distribution, receptor binding, and subsequent rate of metabolic deactivation outlined by Bodor when he conceptualized retrometabolic drug design.

3.2. Clinical Studies: LE Suspension Formulations. Since the design of LE by Bodor and colleagues, 3 ophthalmic suspension formulations of LE have been developed and tested clinically in various ocular inflammatory conditions (Table 1) and postoperative inflammation (Table 2): a 0.2% suspension, a 0.5% suspension, and a combination suspension of LE 0.5% plus tobramycin 0.3%. Clinical safety and efficacy of these formulations is briefly summarized below. These studies confirm the clinical anti-inflammatory potency of LE and lack of significant IOP effects after its use.

Bartlett et al. [29] studied the safety and efficacy of LE 0.5% in the treatment of papillae in contact lens-associated GPC. In this 4-week study, LE-treated patients demonstrated a significant reduction in the primary ocular sign of GPC (papillae, $P \leq 0.02$) and were rated better in the investigator global assessment ($P = 0.017$) as compared to placebo-treated patients. The mean IOP did not change over the course of the study. The efficacy and safety of LE in the management of GPC associated with contact lens use were further evaluated by Asbell and Howes [30] and Friedlaender and Howes [31] in two identical studies. In both studies, patients received 0.5% LE or placebo 4 times daily for 6 weeks. The proportion of patients with an improvement in papillae severity and itching severity was greater in the LE treatment group than in the placebo treatment group ($P \leq 0.001$). A significant improvement in contact lens tolerance in the LE treatment group was observed in 1 study ($P = 0.002$). Transient IOP elevations (≥10 mm Hg from baseline) occurred more often in the LE treatment group but were attributed to the reservoir effect of the contact lens, which patients continued to wear for the duration of the study.

Dell et al. studied the efficacy and safety of 0.5% LE administered prophylactically over a period of 6 weeks before the start of the allergy season in patients with SAC [32]. During peak pollen counts, the results of composite severity of itching and bulbar conjunctival injection and the investigator global assessment significantly favoured LE treatment ($P \leq 0.001$), compared with placebo. An IOP increase of greater than 10 mm Hg was noted in 2 patients receiving placebo and none of the patients treated with LE. The efficacy of LE 0.2% for the treatment of SAC was further evaluated by Dell et al. [33] and Shulman et al. [34] in 2 similar studies. In both studies, LE treatment reduced bulbar conjunctival injection and itching to a greater extent than placebo ($P \leq 0.008$). No patient experienced elevated IOP of ≥10 mm Hg over baseline in one study, while 1 patient in each treatment group experienced an IOP elevation in the second study. Recently,

Table 1: Loteprednol etabonate: summary of randomized, controlled, clinical safety and efficacy studies in ocular Inflammatory diseases.

Ocular disease	Treatment duration and study treatments	Efficacy	Safety	Reference
Giant papillary conjunctivitis	4 weeks LE 0.5% ($n = 55$) versus placebo ($n = 55$)	(i) Reduced papillary severity 1–4 ($P \leq 0.02$ versus placebo) (ii) Investigators global assessment better ($P = 0.017$ versus placebo)	No change in mean IOP in LE treatment group	[29]
	6 weeks LE 0.5% ($n = 111$) versus placebo ($n = 109$)	(i) Reduced papillary severity at final visit ($P < 0.001$ versus placebo) (ii) Reduced itching at final visit ($P = 0.001$ versus placebo) (iii) Improved lens tolerance at final visit ($P = 0.002$ versus placebo)	↑ IOP (≥10 mm Hg): $n = 3$ for LE $n = 0$ for placebo	[30]
	6 weeks LE 0.5% ($n = 109$) versus placebo ($n = 110$)	(i) Reduced papillary severity at final visit ($P = 0.001$ versus placebo) (ii) Reduced itching at final visit ($P < 0.001$ versus placebo) (iii) Improved lens tolerance at final visit ($P = 0.053$ versus placebo)	↑ IOP (≥10 mm Hg): 7% versus 0% $n = 8$ for LE $n = 0$ for placebo	[31]
Prophylaxis of seasonal allergic conjunctivitis	6 weeks LE 0.5% ($n = 145$) versus placebo ($n = 143$)	(i) Reduced composite of itching and BCI ($P = 0.001$ versus placebo) (ii) Investigators global assessment better ($P < 0.001$ versus placebo)	↑ IOP (≥10 mm Hg): $n = 0$ for LE $n = 2$ for placebo	[32]
Seasonal allergic conjunctivitis	6 weeks LE 0.2% ($n = 66$) versus placebo ($n = 67$)	(i) Reduced BCI, itching at 2 weeks ($P \leq 0.034$ versus placebo) (ii) Investigator global assessment at week 2 better ($P < 0.001$ versus placebo)	No ↑ IOP (≥10 mm Hg) ≥1 AE: 68% versus 90% ($P = 0.002$)	[33]
	6 weeks LE 0.2% ($n = 67$) versus placebo ($n = 68$)	(i) Reduced BCI, itching at 2 weeks ($P \leq 0.008$ versus placebo) (ii) Investigator global assessment at week 2 better ($P < 0.001$ versus placebo)	↑ IOP (≥10 mm Hg): $n = 1$ for LE $n = 1$ for placebo No AE: 36% versus 19% ($P = 0.035$)	[34]
	2 weeks LE 0.2% ($n = 151$) versus olopatadine ($n = 149$)	(i) Reduced BCI, itching at week 2 in both groups ($P \leq 0.0006$ in favour of LE)	No ↑ IOP (≥10 mm Hg) ≥ 1 AE: 2.0% versus 1.3% ($P =$ NS)	[35]
Anterior uveitis	6 weeks LE 0.5% ($n = 36$) versus prednisolone 1.0% ($n = 34$)	(i) Resolution of ACC (LOCF): 74% versus 88% ($P =$ NS) (ii) Resolution of flare (LOCF): 71% versus 81% ($P =$ NS) (iii) Resolution of pain (LOCF): 79% versus 81% ($P =$ NS)	↑ IOP (≥10 mm Hg): $n = 0$ for LE $n = 1$ for prednisolone	[36]
	4 weeks LE 0.5% ($n = 84$) versus prednisolone 1.0% ($n = 91$)	(i) Resolution of ACC (LOCF): 72% versus 87% ($P = 0.015$ in favour of prednisolone) (ii) Resolution of flare (LOCF): 66% versus 82% ($P = 0.017$ in favour of prednisolone) (iii) Resolution of pain (LOCF): 90% versus 85% ($P =$ NS)	↑ IOP (≥10 mm Hg): $n = 1$ for LE $n = 6$ for prednisolone	[36]

Table 1: Continued.

Ocular disease	Treatment duration and study treatments	Efficacy	Safety	Reference
Blepharokerato-conjunctivitis	2 weeks LE 0.5%/tobramycin 0.3% ($n = 136$) versus dexamethasone 0.1%/tobramycin 0.3% ($n = 137$)	(i) Improvement from baseline in composite signs and symptoms severity at day 15 in both groups (ii) LE/T noninferior to DM/T in reduced composite signs and symptoms at day 15 (-15.2 [7.3] versus -15.6 [7.7], P = NS) (iii) Investigator global assessment: 43.6% versus 40.9% cured (P = NS)	↑ IOP (≥10 mm Hg): $n = 0$ for LE/T $n = 1$ for DM/T Mean IOP increase at day 15: -0.1 mm Hg versus 1.0 mm Hg ($P = 0.0091$) ≥ 1 AE: 2.9% versus 6.5% (P = NS)	[43]
	2 weeks LE 0.5%/tobramycin 0.03% ($n = 178$) versus dexamethasone 0.1%/tobramycin 0.3% ($n = 176$)	(i) Improvement from baseline in composite signs and symptoms severity at day 15 in both groups ($P < 0.0001$ versus baseline) (ii) LE/T noninferior to DM/T in reduced composite signs and symptoms at day 15 (-11.6 [4.6] versus -12.4 [4.7], P = NS)	↑ IOP (≥10 mm Hg): $n = 6$ for LE/T $n = 13$ for DM/T Mean IOP increase at day 15: 1.33 mm Hg versus 2.43 mm Hg ($P = 0.0039$) ≥1 AE: 13.0% versus 23.2%	[44]
Keratoconjunctivitis sicca	4 weeks 0.5% LE ($n = 32$) versus placebo ($n = 34$)	(i) Reduced hyperaemia at week 2 and week 4 ($P \leq 0.0473$ versus placebo) (ii) Subset analysis in patients with moderate-to-severe inflammation at baseline (iii) Reduced central corneal staining, nasal bulbar conjunctival hyperaemia, and lid margin injection at some visits ($P < 0.05$ versus placebo)	No ↑ IOP (≥10 mm Hg) No significant change in mean IOP ≥1 AE: 16.7% versus 23.5%	[38]

LE: loteprednol etabonate, IOP: intraocular pressure, ACC: anterior chamber cells, AE: adverse event, BCI: bulbar conjunctival injection, LOCF: last observation carried forward, NS: not significant.

Elion-Mboussa et al. [35] compared the clinical efficacy and safety of LE 0.2% with that of an antihistamine, olopatadine 0.1%, in patients with acute SAC. It was found that LE 0.2% was superior to olopatadine in reducing both bulbar injection and ocular itching ($P \leq 0.0006$) following 2 weeks of treatment. No patients experienced a clinically significant increase in IOP (≥10 mm Hg) over baseline, suggesting that the risk of elevated IOP with LE 0.2% may not differ from that with an antihistamine.

Two clinical studies were conducted to compare the efficacy and safety of LE 0.5% to prednisolone acetate 1.0% in the treatment of anterior acute uveitis [36]. In the first study, study treatments were initially administered 8 times daily and continued QID for up to 6 weeks. While in the second study, study treatments were initially administered 16 times a day and continued QID for up to 4 weeks. Both treatments significantly reduced anterior chamber cell and flare as well as pain and photophobia, compared to baseline. However, a last-observation-carried-forward analysis in the second study showed a greater reduction in cell and flare with prednisolone than with LE ($P \leq 0.017$), although no differences were found at any on-treatment study visits. Across the 2 studies, only 1 LE-treated patient versus 7 prednisolone-treated patients experienced an IOP increase of >10 mm Hg over baseline ($P = 0.05$) [37].

LE has also been studied in the treatment of dry eye or keratoconjunctivitis sicca. Pflugfelder et al. conducted a pilot study evaluating the efficacy of LE 0.5% versus placebo for the treatment of patients with dry eyes secondary to delayed tear clearance [38]. Although there were significant within-treatment improvements in the primary subjective variable (visual analogue severity for worst symptom at baseline) in both groups, there were no significant within-treatment improvements in the primary objective variable (composite corneal staining) in either treatment group. Further analysis of a subset of patients with moderate-to-severe inflammation showed a significant difference between the LE-treated group and vehicle-treated group in central corneal staining, nasal bulbar conjunctival hyperaemia, and lid margin injection at some visits ($P < 0.05$). None of the patients

Table 2: Loteprednol etabonate: summary of randomized, controlled, clinical safety and efficacy studies in postoperative inflammation.

Treatment duration and study treatments	Efficacy	Safety	Reference
2 weeks LE 0.5% ($n = 109$) versus placebo ($n = 113$)	(i) Resolution of ACI at final visit: 64% versus 29% ($P < 0.001$ versus placebo) (ii) Treatment failure rate: 6% versus 30% ($P < 0.001$ versus placebo) (iii) Investigator global assessment of treatment effect ($P < 0.001$ versus placebo) (iv) Grade 0 (no pain) at final visit: 85% versus 54% ($P = 0.003$)	↑ IOP (≥10 mm Hg) $n = 3$ for LE $n = 0$ for placebo Mean IOP decreased in both groups ≥1 AE: 58% versus 80% ($P < 0.001$)	[41, 42]
2 weeks LE 0.5% ($n = 102$) versus placebo ($n = 101$)	(i) Resolution of ACI at final visit: 55% versus 28% ($P < 0.001$) (ii) Treatment failure rate: 7% versus 32% ($P < 0.001$ versus placebo) (iii) Investigator global assessment of treatment effect ($P < 0.001$ versus placebo) (iv) Grade 0 (no pain) at final visit: 83% versus 59% ($P = 0.018$)	↑ IOP ≥10 mm Hg) $n = 0$ for LE $n = 1$ for placebo Mean IOP decreased in both groups ≥1 AE: 54% versus 75% ($P = 0.002$)	[6, 42]
2 weeks LE 0.5% ointment ($n = 404$) versus vehicle ($n = 401$) [2 studies]	(i) Resolution of ACI at day 8: 27.7% versus 12.5% ($P < 0.0001$) (ii) Grade 0 (no pain) at day 8: 75.5% versus 43.1% ($P < 0.0001$) (iii) Need for rescue medication: 27.7% versus 63.8% ($P < 0.0001$)	↑ IOP (≥10 mm Hg): $n = 3$ for LE $n = 1$ for vehicle Mean IOP decreased in both groups Mean IOP decreased in both groups ≥1 AE: 47.2% versus 78.0% ($P < 0.0001$)	[45]

LE: loteprednol etabonate, IOP: intraocular pressure, ACI: anterior chamber inflammation, AE: adverse event.

experienced a clinically significant increase in IOP following 1 month of therapy. LE 0.5% has also been studied as induction therapy for topical cyclosporine ophthalmic emulsion 0.05% in the treatment of patients with dry eye [39]. Cyclosporine improves tear production in patients with ocular inflammation associated with dry eye. However, relief of signs and symptoms is often delayed by 1 to 6 months from the initiation of therapy, and it has been reported that 1 in 5 patients treated with cyclosporine experiences burning and stinging. LE induction therapy administered 2–6 months prior to the institution of long-term cyclosporine treatment decreased stinging and improved compliance when compared with the cohort of patients who were prescribed cyclosporine without LE induction therapy ($P \leq 0.04$). A follow-up study presented in abstract form indicated that 2 weeks of induction therapy with LE was sufficient to improve subjective and objective parameters, compared to artificial tears alone, thereby accelerating clinical improvement [40].

Two identical placebo-controlled trials examined the safety and efficacy of LE in treating postoperative inflammation following cataract surgery with intraocular lens implantation [6, 41]. Patients were administered 1 drop of LE 0.5% or vehicle in each eye every 4 hours, 4 times daily for up to 14 days. In both studies, greater resolution of anterior chamber inflammation (the sum of anterior chamber cells and flare) was achieved with LE than with placebo ($P < 0.001$). Results for pain resolution, reported separately, [42] indicated that 84% of LE-treated patients, compared to 56% of vehicle-treated patients, across the 2 studies had no pain at the final visit ($P < 0.05$). The mean IOP decreased after surgery in both the LE and placebo treatment groups.

The combination of LE 0.5% and tobramycin 0.3% (LE/T) was evaluated in the treatment of blepharokeratoconjunctivitis (BKC) in 2 studies [43, 44]. Both White et al. and Chen et al. compared the safety and efficacy of LE/T with that of dexamethasone 0.1%/tobramycin 0.3% (DM/T). Subjects in each study were randomized to LE/T or DM/T administered 4 times daily for 14 days. Both steroid combinations were effective in improving the signs and symptoms of BKC relative to baseline ($P \leq 0.0001$). In both studies, there were no significant differences in the mean change from baseline to day 15 in the signs and symptoms of composite severity, and LE/T was found to be noninferior to DM/T. However, in both studies, DM/T-treated patients experienced a significant increase in the mean IOP when compared with LE/T-treated patients ($P \leq 0.0339$). IOP increases of ≥10 mm Hg over baseline were reported more often for the DM/T treatment group.

3.3. New Formulations of Loteprednol Etabonate. The safety and efficacy of LE ophthalmic ointment 0.5% (LE ointment) in the treatment of inflammation and pain following cataract surgery were studied in 2 randomized, multicentre, double-masked, parallel-group, vehicle-controlled studies [45]. Pooled analysis of the data from these studies showed that significantly more LE ointment-treated patients than vehicle-treated patients had complete resolution of anterior chamber inflammation and no pain at day 8 of treatment ($P < 0.0001$). Fewer LE ointment-treated patients required rescue medication, and fewer had an ocular adverse event.

Studies are also underway on a new gel formulation of LE 0.5% in the treatment of inflammation and pain following cataract surgery (NCT01010633 and NCT01060072). As indicated previously, LE is highly lipophilic with limited solubility in water. A gel formulation could provide improved product homogeneity over a suspension formulation and perhaps a more consistent clinical response as a consequence. Results of these studies are expected to be released in 2012.

4. IOP and Cataract Formation with Loteprednol Etabonate

The clinical studies summarized above confirm the efficacy of LE in the treatment of ocular inflammatory disease and postoperative inflammation associated with cataract surgery and are supportive of LE meeting the required balance between the solubility/lipophilicity, ocular tissue distribution, receptor binding, and subsequent rate of metabolic deactivation, all of which are essential features of successful retrometabolic design. Additional studies with LE, including studies in known steroid responders, and additional study analyses further confirm the reduced incidence of ADRs with LE in clinical practice.

Holland et al. [46] compared the steroid-induced IOP effect and other ocular adverse effects of LE/T with those of DM/T in 306 healthy volunteers. In this study, patients were treated 4 times daily for 28 days or longer. The number of patients experiencing IOP increases of ≥10 mm Hg from baseline at any study visit was significantly lower in the LE/T group than in the DM/T group (1.95% versus 7.48%; $P = 0.028$); similar results were observed for mean changes from baseline in IOP ($P < 0.05$ at all visits). Patients in the LE/T group were also more likely to report better ocular comfort/tolerability ratings relative to an artificial tear standard, compared to subjects in the DM/T group [47].

Novack et al. [48] conducted a meta-analysis of the IOP data from LE development studies in which patients were treated with LE, of any concentration, for 28 days or longer. The analysis included a combination of 1648 healthy volunteers and patients with a variety of ocular inflammatory conditions. IOP elevations of ≥10 mm Hg over baseline occurred in 1.7% (15/901) patients using LE, compared to 0.5% (3/583) patients using vehicle and 6.7% (11/164) patients using prednisolone acetate. Excluding subjects that continued to wear soft contact lenses (allowed in the GPC trials and thought to contribute to a reservoir effect), the rates were 0.6%, 1.0%, and 6.7% for LE, vehicle, and prednisolone acetate, respectively. Cheng et al. also conducted a meta-analysis of LE IOP data but included data retrieved from available published LE clinical studies [37]. A total of 1660 patients with a variety of ocular conditions were included in this analysis. In placebo-controlled studies, the IOP elevation rate was 1.7% in the LE group versus 0.6% in the placebo group ($P = 0.3$). In active (prednisolone) comparator studies, the IOP elevation rate was 0.8% in the LE group versus 5.5% in the prednisolone group ($P = 0.05$).

The absence of significant ADRs was further studied by Ilyas et al. who studied the long-term safety of LE 0.2% by conducting a retrospective review of 397 seasonal and perennial conjunctivitis patients who had used LE 0.2% on a daily basis for extended periods of time [49]. Of these patients, 159 had been using LE 0.2% continuously for at least 12 months. There were no reports of posterior subcapsular opacification and no clinically meaningful changes in IOP in this group. In fact, there were no observations of IOP elevations greater than 4 mm Hg over baseline at any time.

Bartlett et al. [50] compared the effects of LE 0.5% and prednisolone acetate 1.0% on IOP in a crossover study in 19 known steroid responders. Studies in known steroids responders are useful since differences in steroid-induced IOP effects are emphasized in this population. Subjects received either LE 0.5% or prednisolone 1.0% for 42 days followed by a washout period of 14 days prior to being crossed over to the other treatment. During LE treatment, the mean IOPs were within the normal range, with a mean IOP elevation of 4.1 mm Hg over the 42-day period (P, not significant). In contrast, during prednisolone treatment, the mean IOP elevation was 9.0 mm Hg ($P < 0.05$, compared to baseline) (Figure 5). Because the study protocol required discontinuation of subjects upon significant IOP elevation, the authors noted that the hypertensive effect of prednisolone may have been underestimated.

Finally, Holland et al. [51] reported the attenuation of ocular hypertension in steroid responders after corneal transplantation. In this retrospective review, 30 post-penetrating keratoplasty and post-keratolimbal allograft patients with IOP increases to ≥21 mm Hg while being treated with prednisolone acetate 1.0% were switched to LE 0.5%. Results showed a mean (SE) reduction of IOP from 31.1 (1.13) mm Hg to 18.2 (1.37) mm Hg ($P = 0.0001$) with no signs of graft rejection after switching treatment from prednisolone acetate to LE.

With respect to cataract formation, as indicated earlier, Manabe et al. showed that C-20 ketone steroids such as prednisolone form covalent bonds with lens protein. These authors further showed that nonketolic analogues were unable to form such adducts. Bodor and colleagues designed LE with a C-20 ester rather than a C-20 ketone, and thus LE is unable to form covalent adducts via this mechanism, although other mechanisms of cataractogenesis cannot be ruled out. Nevertheless, the long-term study by Ilyas et al. did not suggest a potential for cataract formation with LE. Further, a review of global postmarketing adverse event data for LE 0.5% revealed only 7 reports of cataract formation with LE use (data through August 2011, Bausch & Lomb, data on file) since product launch. During that time, an estimated 20

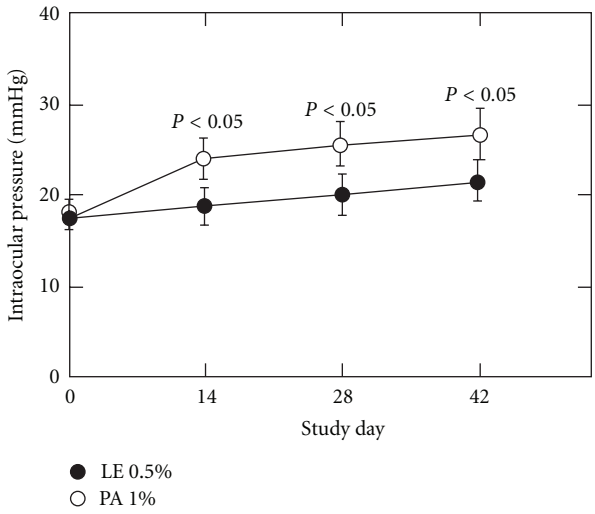


Figure 5: Mean (SEM) IOP for subjects receiving loteprednol etabonate and prednisolone. Within-treatment significant changes from baseline are indicated adapted from [50].

million LE units were distributed globally. Taken together, these data suggest that the rapid metabolism of LE to inactive hydrophilic metabolites in conjunction with the lack of the C-20 ketone have resulted in a steroid with significantly less, if any, potential for promoting cataract formation.

5. Conclusions

Retrometabolic drug design principles have led to the development of LE, a C-20 ester corticosteroid. LE appears to achieve the necessary balance between solubility/lipophilicity, tissue distribution, GR receptor binding, and metabolic deactivation to be effective as a topical ophthalmic steroid. LE is safe and effective in treating a wide variety of ocular inflammatory conditions including giant papillary conjunctivitis, seasonal allergic conjunctivitis, and uveitis as well as in the treatment of ocular inflammation and pain following cataract surgery. ADRs such as cataract formation and IOP elevation were minimized with LE owing to its retrometabolic design and their absence confirmed in clinical studies.

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Review Article

Renin-Angiotensin System Hyperactivation Can Induce Inflammation and Retinal Neural Dysfunction

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The renin-angiotensin system (RAS) is a hormone system that has been classically known as a blood pressure regulator but is becoming well recognized as a proinflammatory mediator. In many diverse tissues, RAS pathway elements are also produced intrinsically, making it possible for tissues to respond more dynamically to systemic or local cues. While RAS is important for controlling normal inflammatory responses, hyperactivation of the pathway can cause neural dysfunction by inducing accelerated degradation of some neuronal proteins such as synaptophysin and by activating pathological glial responses. Chronic inflammation and oxidative stress are risk factors for high incidence vision-threatening diseases such as diabetic retinopathy (DR), age-related macular degeneration (AMD), and glaucoma. In fact, increasing evidence suggests that RAS inhibition may actually prevent progression of various ocular diseases including uveitis, DR, AMD, and glaucoma. Therefore, RAS inhibition may be a promising therapeutic approach to fine-tune inflammatory responses and to prevent or treat certain ocular and neurodegenerative diseases.

1. Introduction

Most visual disorders occur in the retina, which is a part of the central nervous system (CNS) and consists of neurons, glia, pigment epithelium (RPE), and blood vessels. Currently, diabetic retinopathy (DR), age-related macular degeneration (AMD), and glaucoma are the top causes of blindness in the developed countries. These diseases can occur when local or systemic neuronal and vascular homeostasis mechanisms are dysregulated. The highest risk factor for many of these diseases is aging [1–3], and as is the case with other age-related diseases such as Alzheimer's disease, cardiovascular disease, cancer, arthritis, osteoporosis, and hypertension, accumulating evidence suggests that chronic inflammation and oxidative stress can accelerate or promote disease progression [4–6].

The renin-angiotensin system (RAS) is classically known as a systemic blood-pressure-regulating system. However, it is becoming widely recognized as an inflammation regulator as well. Independent of systemic RAS, tissue intrinsic RASs have been identified in various tissues (including the retina) and are important for maintaining local homeostasis. Elements of the RAS pathway are highly conserved in many species including invertebrates and humans demonstrating that its functions are evolutionarily conserved, although spatial expression patterns differ slightly between different species [7].

We have reported that angiotensin II type 1 receptor blocker (ARB) suppresses retinal neural dysfunction in animal models of acute inflammation [8] or diabetes [9]. Other groups and our own have also reported that ARBs can protect retinal vascular inflammation [10–19] and neuronal

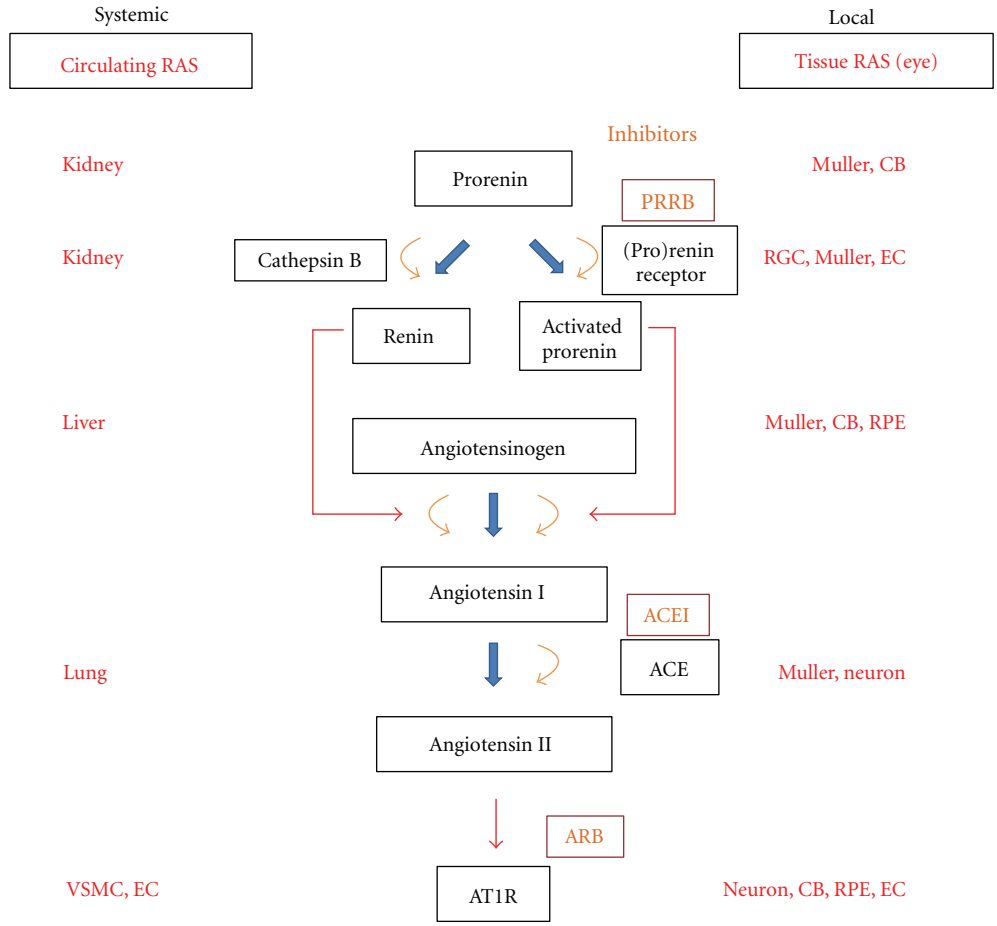

Figure 1: Circulating and tissue renin-angiotensin system (RAS). VSMC: vascular smooth muscle cell, EC: endothelial cell, PRRB: (pro)renin receptor blockers, ACEI: angiotensin-converting enzyme inhibitors, ARB: angiotensin II type 1 receptor blockers, AT1R: angiotensin II type 1 receptor, CB: ciliary body, RPE: retinal pigment epithelium.

apoptosis [20–23]. Furthermore, it was recently reported by two independent groups that daily oral administration of ARB may prevent the progression of diabetic retinopathy in randomized multicenter clinical trials [24–26]. In this paper we will summarize these findings and other studies demonstrating that RAS modulation may prevent ocular pathogenesis. We will also outline the similarities and differences between retinal and brain RAS. Lastly, we will describe the potential mechanisms through which RAS inhibition may preserve neuronal function and viability while combating ocular diseases.

2. RAS as an Inflammatory Cascade

Renin was discovered as a hypertensive agent in rabbit kidneys in 1898. It was later found to induce the release of a vasoconstrictive agent in experimental models of hypertension induced by renal ischemia [27]. Two independent groups identified the end product of this hypertensive cascade in 1939 and named it "hypertension" [28] or "angiotonin" [29]. It has since been renamed "angiotensin" [30]. The RAS pathway as we know it today began to take shape once angiotensin-converting enzyme (ACE) was identified in 1956 [31]. We now know that once renin is proteolytically processed from its precursor prorenin by proteases and released from the kidney, it converts angiotensinogen to angiotensin I in the liver. Angiotensin I is finally converted to angiotensin II by ACE which is predominantly expressed in vascular endothelial cells (ECs) and is located in highly vascularized tissues such as the lung (Figure 1). Angiotensin II stimulates vascular smooth muscle cells (VSMCs) that line endothelial cells to contract and induce vasoconstriction.

There are two primary receptors for angiotensin II: angiotensin II type 1 receptor (AT1R) and AT2R; AT1R appears to exert predominant functions in blood vessels. Generally, AT1R signaling normally induces vasoconstriction while AT2R signaling induces vasodilation. However, the roles of AT1R and AT2R in pathophysiological conditions are currently under debate [32–34]. AT1R is a seven-transmembrane G protein-coupled receptor [35, 36]. Once stimulated in VSMCs G proteins activate phospholipase C (PLC) and inositol-1,4,5-triphosphate (IP3) to open calcium channels in the endoplasmic reticulum [37]. As a result, increase of

cytosolic calcium induces phosphorylation of myosin light chain, VSMC contraction, and vasoconstriction [38, 39].

Independent of systemically circulating angiotensin II (circulating RAS), most RAS components, including ACE, were also found to be locally expressed in many tissues [40]. This observation resulted in the hypothesis that in addition to being converted in particular organs for systemic circulation, angiotensin II could also be synthesized in peripheral tissues (tissue RAS) where it would exert its effect locally. Tissue RAS elements were identified in various organs including heart [41], kidney [42], adrenal gland [43], brain [44], and retina (see details below). An important molecule involved with tissue RAS is (pro)renin receptor which interacts with prorenin to exert enzymatic activity of renin without the conventional proteolysis of the prorenin prosegment [45, 46]. (Pro)renin receptor can be detected in major organs but not in circulation indicating that this molecule may play a critical role in the activation of tissue RAS [46]. Thus tissue RAS may be important for fine-tuning global RAS activity or for acting intrinsically to respond to localized insults. However, (pro)renin receptor may also function independent of renin activation as a member of the Wnt receptor complex to regulate Wnt/ß-catenin pathway activity [47].

In addition to its critical physiological functions, RAS dysregulation can lead to pathogenesis. In various cardiovascular cell-type RASs hyperactivation can induce pathogenic cell migration, hypertrophy, fibrosis, disrupt cell adhesion and ectopic extracellular matrix (ECM) deposition. AT1R signaling directly activates key signaling pathways for cell growth and hypertrophy including JAK/STAT (janus kinase/signal transducer and activator of transcription) [48, 49], ERK (extracellular-signal-regulated kinase) 1/2 [50–52], and p38 MAPK (mitogen-activated protein kinase) [53]. Indeed, angiotensin II/AT1R signaling can potentiate oxidative stresses and inflammatory responses by activation of NAD(P)H (nicotinamide adenine dinucleotide phosphate) oxidases [54–57]. Angiotensin II can also activate EGFR (epidermal growth factor receptors) and induces fibronectin synthesis and TGFβ (transforming growth factor beta) activity to promote fibrosis and ECM formation [58, 59]. AT1R signaling can activate NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells) [60–62] and AP-1 (activator protein 1) to initiate transcription of multiple proinflammatory genes [61, 63, 64]. AT1R also induces accumulation, adhesion, and infiltration of inflammatory cells via activation of PAI-1 (plasminogen activator inhibitor-1) [65] and MCP-1 (monocyte chemotactic protein-1) [62] to promote atherosclerosis [66]. Taken together, these findings provide strong evidence that RAS is not just a regulator of blood pressure, but also regulates an inflammatory cascade.

The effects of circulating and tissue RAS can be controlled with RAS inhibitors. After the first ACE inhibitor (ACEI) was developed [67], many other RAS inhibitors including ARB [68, 69] have been established and approved for commercial use as hypertension drugs (Figure 1). RAS inhibition not only prevents hypertension but also protects tissues against injury by limiting the potency of deleterious inflammatory responses. Since aging is considered to be, in part, the result of chronic inflammation [70], it may not be too surprising that the use of RAS inhibitors or genetic deletion of AT1R has potential to extend the life span in hypertensive [71–73] or normotensive [74] mammals.

3. Brain and the Retinal RAS

In addition to regulating vasoconstriction, another important physiological function of RAS is osmoregulation in the CNS (e.g., water and sodium intake, sympathetic activity, and release of vasopressin) [75–77]. AT1R is expressed in brain neurons and mediates osmoregulation [76] by stimulating the release of vasopressin in the pituitary gland and signaling the kidney to conserve water [76]. Furthermore angiotensin II/AT1R signaling in the brain forces individuals to stimulate increased thirst and consume more drinking water [77]. Since angiotensin II has a high molecular weight, it does not cross the blood-brain barrier (BBB) [78]. Therefore intrinsic RAS networks must be responsible for inducing the dipsogenic activity. Additionally, every component of the RAS pathway including angiotensinogen, ACE, and angiotensin II receptors is expressed in the brain [75, 76, 79–81]. Brain RAS can also become dysregulated; this has been shown to induce oxidative stress and inflammation [82]. However, RAS inhibitors have neuroprotective effects in brain inflammation and ischemia without inducing antihypertension (see detail below).

The retina also has an intrinsic tissue RAS. In the eye, prorenin protein and renin activity can be detected in the vitreous fluid [83–85] and prorenin mRNA has been detected in Muller glia [86] and in the ciliary body (CB) cells [87]. (Pro)renin receptor is expressed in ECs, Muller glia, and retinal ganglion cells (RGCs) [88, 89]. Angiotensinogen is found in CB [90], Muller glia [91], and RPE [92]. ACE is synthesized in the neural retina [93, 94] and can be detected in RGCs, photoreceptors [95], and Muller glia [96]. Angiotensin II, the final product of RAS, can be detected in the vitreous fluid [97] and in the neural retina [98]. Interestingly, the normal concentration of angiotensin II in ocular fluid is higher than in plasma [97], confirming the existence of tissue RAS in the eye.

In the retina, angiotensin II receptors are detected both in ECs and in neuronal cells, which are located outside and inside of the blood-retina barrier (BRB), respectively [8, 92, 99, 100]. AT1R is found in the presynaptic terminals of photoreceptors and of interneurons in the retina [8] as well as in neurons of the brain [101, 102] (Figure 2). AT1R is also expressed in RGCs [103], although the physiological function of AT1R in the neural retina is not fully understood. Systemic administration of ACEI negatively influences cat and human neural functions measured by electroretinograms (ERG) in both systemic blood-pressure-dependent [104] and -independent manners [105, 106]. Additionally, angiotensin II increases voltage-dependent calcium currents in cultured RGCs [103]. Therefore ocular RAS may act as a physiological neuromodulator.

AT2R is also expressed in the retina [8] but much less is known how it functions in the eye. Polymorphisms in the AT2R gene may be linked to glaucoma [107] or diameter of the retinal arterioles [108].

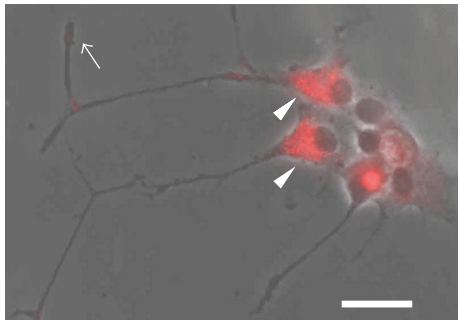

Figure 2: AT1R expression in a neuronal cell line. Immunohistochemistry for angiotensin II type 1 receptor (AT1R) in PC12D cells. Note that AT1R is detected in presynaptic terminal (arrow) or soma (arrow head). Scale bar: 20 μm.

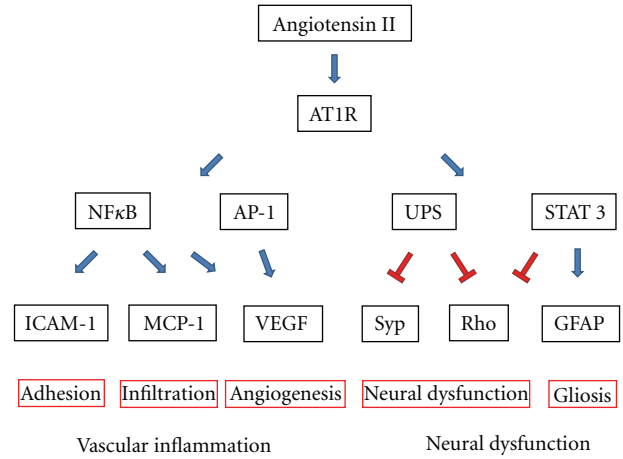

Figure 3: Downstream of AT1R in vascular inflammation and neural dysfunction. NFκB: nuclear factor kappa-light-chain-enhancer of activated B cells, AP-1: activator protein 1, UPS: ubiquitin-proteasome system, STAT3: signal transducer and activator of transcription 3, ICAM-1: intercellular adhesion molecule 1, MCP-1: monocyte chemotactic protein 1, VEGF: vascular endothelial growth factor, Syp: synaptophysin, Rho: rhodopsin, GFAP: glial fibrillary acidic protein.

4. RAS and Ocular Diseases

4.1. Uveitis. Increasing evidence suggests that RAS activity and inflammation may be associated with various ocular diseases, and, therefore, RAS inhibitors may be effective therapeutic agents. Several lines of evidences suggest that RAS inhibition is an effective treatment for uveitis [8, 12, 17, 18, 88]. Endotoxin-induced uveitis (EIU) is induced with intraperitoneal injections of lipopolysaccharide (LPS); this results in upregulated expression of proinflammatory and adhesion molecules such as ICAM-1 (intercellular adhesion molecule 1), MCP-1, IL-6 (interleukin 6), and IFN-γ (interferon-gamma) [17, 88]. These molecules are also upregulated in experimental autoimmune uveoretinitis (EAU) models generated by immunizing animals with interphotoreceptor retinoid-binding protein (IRBP) [18]. The upregulation of these molecules, however, can be inhibited with ARB or (pro)renin receptor blocker (PRRB). (PRRB is an experimental decoy peptide that contains "handle" region sequence of (pro)renin receptor.) RAS inhibition also suppresses retinal leukocyte stasis, CD4+ T-cell activation [17, 18, 88]. Furthermore, RAS inhibition suppresses gliosis by preventing STAT3 activation [8]. Lastly, when the expression levels of RAS pathway components are examined in EIU, prorenin, (pro)renin receptor [88], angiotensin II [8], and AT1R [17] levels are elevated in the retina. These findings suggest that heightened inflammatory responses in the eye and RAS activation are strongly correlated.

4.2. Chronic Inflammation and Eye Diseases. Besides being correlated with classically acute inflammation cases such as uveitis, one of the largest risk factors for developing prevalent and vision-threatening diseases such as DR, AMD, and glaucoma is aging [1–3]. These age-related eye diseases [109, 110] and others [5, 6] are now known to be caused (at least partially) by chronic inflammation and oxidative stress. Since RAS inhibition may prolong the life spans of hypertensive [71–73] or normotensive [74] mammals, it is logical that age-related eye diseases may be prevented or treated by suppressing inflammation and oxidative stress. The main pathological event of DR and AMD is abnormal neovascularization and VEGF (vascular endothelial growth factor) has been known to be a large contri-butor for them [111–113]. VEGF is a potent angiogenic factor and an inflammatory cytokine that induces the accumulation, adhesion, and infiltration of leukocytes [114, 115]. Inflammatory response in the retina can promote tissue ischemia by inducing vascular regression (vaso-obliteration) and also pathological angiogenesis [116]. Angiotensin II can induce upregulation of VEGF receptor (VEGFR)-2 and angiopoietin-2 in retinal ECs [117, 118] and VEGF in retinal pericytes [119] (Figure 3). Oxygen-induced retinopathy (OIR) is an animal model induced by continual aeration with 75–80% oxygen in early postnatal stages. OIR animals develop stereotypical phenotypes and is useful to evaluate vaso-obliteration and pathological angiogenesis (tuft formation) in the developing retina [120] which is largely re-gulated by VEGF [121]. This phenotype can be prevented with RAS inhibitors ACEI [122, 123], ARB [15], or PRRB [89, 124] that prevent pathological angiogenesis in OIR. The use of ARB and PRRB has the added benefit of suppressing abnormal angiogenesis without suppressing physiological vascular regeneration [15, 124]. In animals exposed to OIR RAS inhibitors may function to prevent gene expression of proinflammatory molecules and prevent leukocyte infiltration. Infiltration of VEGF-expressing inflammatory cells into the vitreous cavity is thought to induce pathological angiogenesis by causing ECs to grow in the wrong direction [115].

4.3. Diabetic Retinopathy (DR). DR is one of the leading causes of blindness in the world [3]. It is characterized by vascular loss due to hyperglycemia and inflammation due to oxidative stress and AGEs (advanced glycation end products) accumulation. In severe cases hypoxia induces abnormal neovascularization (proliferative diabetic retinopathy, PDR) in addition to hyperpermeability (diabetic macular edema; DME). Prorenin [83] and angiotensin II [125, 126] are found

to be increased in the vitreous humor of PDR and DR patients. RAS may potentiate the vascular phenotype of DR by upregulating VEGF/VEGFR-2 signaling (through angiotensin II) [118, 119] thereby inducing neovascularization and promoting blood vessel permeability. In fact, VEGF was initially named "vascular permeability factor" (VPF) [127].

Multiple attempts have been made to treat DR with RAS inhibitors. Although in one study ACEI administration seemed to attenuate retinal hyperpermeability in diabetic patients [128], interpretations of these studies are still being actively debated [129, 130]. However, recently three independent groups showed that ARB prevents BRB breakdown in animal models [131–133]. In 1998 and 2008, the results of randomized double-blind placebo-controlled trials using ACEI or ARB to treat DR were released from the EUCLID (EURODIAB Controlled Trial of Lisinopril in Insulin-Dependent Diabetes; ACEI treatment) [134] and DIRECT (Diabetic Retinopathy Candesartan Trial; ARB treatment) [24, 25]. Afterwards, RASS (Renin-Angiotensin System Study) in which both inhibitors were tested in DR patients was also released [26]. Large number of participants were examined in these trials (354 (type 1 diabetes) for EUCLID, 1421 (type 1) and 1905 (type 2) for DIRECT, and 285 (type 1) for RASS, resp.), and the results from all three studies provided strong evidence that RAS inhibition delays the onset or prevents the development of human DR symptom. However, these treatments were not universally beneficial. For example, in DIRECT, ARBs were not effective with respect to primary endpoints and had differing effects regarding secondary endpoints in different patient groups (type I or type II diabetes) [24, 25].

Clues for why RAS inhibition is effective for treating DR have come from animal studies. Streptozotocin (STZ) injections in rodents induce leukocyte stasis, blood vessel hyperpermeability, and formation of acellular capillaries. Importantly, ERG recordings are attenuated in rodents after STZ injections before vascular phenotypes are observed, indicating that neuronal dysfunction precedes neovascularization in diabetic models [9, 135]. Apoptosis of retinal neurons is also observed in later stage [136]. The administration of ACEI [137–140], ARB [10, 13, 14, 141], or PRRB [142] has been shown to rescue the vascular phenotypes of STZ-induced diabetic retinas. To generate another and more severe model of DR, Ren-2 transgenic rats (that have severe hypertension due to genetic knock-in of a mouse ren-2 renin gene [143]) can be injected with STZ. In these rats advanced vascular phenotypes are observed (including abnormal endothelial proliferation). Even in this model ACEI [144] or ARB [19, 145, 146] administration served as effective treatments. RAS inhibitors probably function by suppressing inflammatory cascades [10, 14] and by preventing oxidative stress [147] by limiting NFκB and NAD(P)H activation. RAS inhibitors may also function to directly inhibit glucose accumulation into retinal cells by modulating GLUT-1 (glucose transporter 1) expression [148]. Furthermore, ARB was reported to influence the expression of glyoxalase I, a key regulator of AGEs [11]. Lastly, even though AT1R and AT2R are considered to have opposing functions AT2R inhibition may also effectively treat DR by suppressing VEGF and angiopoietin-2 expression levels in experimental retinopathies [33, 149].

4.4. Age-Related Macular Degeneration (AMD). AMD is one of the leading causes of blindness especially in western countries. The greatest risk factors are aging and smoking [1], and the central phenotypes are choroidal neovascularization (CNV; wet AMD) and atrophy of photoreceptors and RPE cells (dry AMD). While no cure exists for dry AMD, wet AMD is currently treated with VEGF inhibitors [112, 113]. Inflammation exacerbates the wet AMD phenotype since infiltrating macrophages promote CNV formation [150–152]. Experimental CNV can be induced using laser coagulation to mechanically disrupt Bruch's membrane. The size of the laser-induced lesions after treatment with ACEI [153], ARB [16], and PRRB [154] is significantly reduced. Furthermore, AT1R-deficient mice are resistant to laser-induced CNV [154]. RAS inhibition may protect against CNV formation by inhibiting RAS activity and suppressing ERK signaling (directly with (pro)renin receptor-mediated intracellular signaling) [154].

RPE cells are positioned between the choroidal vasculature and photoreceptors and have function to maintain the visual (retinoid) cycle and to form a tight seal that prevents choroidal vessel invasion. Angiotensin II signaling in RPE cells increases abnormal production [155–157] and excessive turnover [158] of ECM via MMP (matrix metalloproteinase)-2 and -14 thereby weakening the seal that prevent choroidal EC invasion. These studies suggest that RAS inhibition may be an effective treatment for AMD as well as DR.

4.5. Glaucoma. Glaucoma is another age-related and high incidence ocular disease [2]. The feature of this disease is neurodegenerative of RGCs, but it can be caused by heterogeneous and complex mechanisms. One direct mechanism to induce RGC death is to increase the intraocular pressure (IOP). Studies devoted to developing new methods of controlling IOP are critical and ongoing. However, a subpopulation of glaucoma patients have normal IOP (normal tension glaucoma, NTG). This complicates the development of effective therapies since both forms are induced by seemingly separate mechanisms. Some RAS components including angiotensin II receptors are expressed in CB cells [90, 159, 160] that secrete aqueous humor and regulate IOP. Like other antihypertensive drugs such as calcium channel blockers, ACEI or ARB decreases IOP in humans and other primates [161–165] although IOP is considered to be regulated independently of systemic blood pressure. In an experimental model of high IOP and glaucoma, ARB treatments effectively suppress RGC death [23]. These findings suggest that RAS inhibition may be effective for treating glaucoma patients with high IOP.

5. RAS Inhibition Protects Brain and Retinal Neurons

Angiotensin II receptors are expressed inside and outside of the BBB [75, 76, 79–81] and the BRB [8, 92, 99, 100] indicating that both circulating and tissue RAS exist in the CNS,

and if dysregulated, could elicit pathological effects. Indeed, RAS inhibition can attenuate the degree of inflammation in the brain and the eye [166, 167]. Inhibiting RAS can prevent experimental brain injuries induced by middle cerebral artery occlusion [168, 169] by suppressing vascular inflammation [170], including BBB breakdown [171], and/or regulating neural apoptosis directly [169]. Interestingly, AT2R is more highly expressed in developing neuronal tissues in vivo than in adult tissues [172] and AT2R stimulation promotes axonal regeneration of optic nerve [173] and minimizes formation of ischemia-induced cerebral lesions [174]. This suggests that ARB, which not only blocks AT1R but also causes angiotensin II to bind AT2R [175], may be an ideal drug for treating inflammatory diseases in the CNS. Inhibition of RAS may also prevent stress-induced behaviors including anxiety, depression, and panic by suppressing the release of corticotrophin-releasing factor [176–178]. Furthermore, recent studies suggest that brain RAS may potentiate Alzheimer's disease progression by stimulating the production of beta amyloid [179–182].

Retinal dysfunction as detected in ERG recordings can be observed in early diabetic animal models and in humans before vascular changes and neural cell loss are observed [135]. Amazingly, these deficits can be prevented by inhibiting RAS [9, 183, 184]. We have reported that ARB prevents retinal dysfunction (e.g., decrease of amplitude and an extension of the implicit time of ERG) in EIU [8] and in STZ-induced early diabetic retinas [9]. Furthermore, in these inflamed retinas, we determined that angiotensin II prompted the degradation of the presynaptic protein synaptophysin through the ubiquitin proteasome system (UPS) [8, 9]. UPS-mediated degradation of rhodopsin (part of the light-responsive complex in photoreceptors) can also be observed in EIU via STAT3 activation (which operates downstream of AT1R) [8, 185]. Additionally, STAT3 signaling serves as a negative regulator of rhodopsin in differentiating photoreceptors during retinal development [186, 187]. Thus, regulating angiotensin-II-induced protein degradation could serve as an important neuroprotective measure [188] (Figure 3).

Another target of inflammation is reactive glia including microglia, astrocytes, and Muller glia. Activated glia cause gliosis and alter proper neuronal morphology. Microglia are resident CNS myeloid-derived cells and mediate critical immune and inflammatory responses. AT1R signaling induces activation of microglia via NFκB and AP-1 [189, 190]. GFAP (glial fibrillary acidic protein) is a differential and reactive marker of astrocyte and Muller glia, respectively, and its transcription is regulated by STAT3 activation [191]. The activation of astrocytes and Muller glia in experimental retinopathy can be prevented by ARB [8, 192] (Figure 3), although it is important to consider that the contributions of reactive glia can be context dependent [193].

IOP-independent RGC apoptosis can be observed in STZ-induced diabetes [136], after ischemia/reperfusion [194], after optic nerve crush [195], and after intraocular NMDA (*N*-methyl-D-aspartic acid) injections [196] in animal models. RGC loss in diabetic hypertensive models can be prevented by ARB which restores oxidative redox and mitochondrial functions [22]. ACEI or ARB also prevents RGC apoptosis in ischemia/reperfusion models by suppressing toxic oxidative stress [21]. ARB can also rescue dying amacrine cells in OIR [20]. Polymorphisms of RAS pathway genes are reported to be associated with brain infarction or its early lesion [197–199] and AT2R gene polymorphisms are reported to be associated with the risk of NTG [107]. These findings may indicate that RAS inhibitors may directly protect retinal neurons from apoptosis and further suggest that RAS inhibition may be useful for therapeutic treatments of IOP-independent glaucoma.

6. Conclusion

RAS, which has been classically known as blood pressure regulator, is becoming widely recognized as a proinflammatory mediator. Many age-related ocular diseases may be caused or exacerbated by chronic inflammation. Cells in the eye are responsive to circulating and tissue RAS and increasing evidence indicates that RAS inhibition may prevent various ocular diseases including uveitis, AMD, and glaucoma. Based on the findings from multiple clinical trials, RAS inhibitors are effective therapeutic agents for treating DR although the results of these studies must be examined critically since the inhibitors were not universally beneficial. Other groups including our own have shown that RAS inhibitors protect neurons from oxidative stress and apoptosis by preventing posttranslational ubiquitination of proteins critical for retinal functions. Although not mentioned previously in this paper, another new and exciting RAS inhibitor, aliskiren (a direct renin inhibitor), has been developed. It may actually mediate more robust vascular protection than either ACEI or ARB [200]. Therefore, work is underway to characterize existing RAS inhibitors and to develop novel inhibitors since they hold great promise for attenuating chronic inflammation and for treating multiple ocular and nonocular diseases.

Abbreviations List (In Order of Their Appearance)

RAS: Renin-angiotensin system
DR: Diabetic retinopathy
AMD: Age-related macular degeneration
CNS: Central nervous system
RPE: Retinal pigment epithelium
ARB: Angiotensin II type 1 receptor blocker
ACE: Angiotensin-converting enzyme
EC: Vascular endothelial cell
VSMC: Vascular smooth muscle cell
AT1R: Angiotensin II type 1 receptor
IP3: Inositol-1,4,5-triphosphate
PLC: Phospholipase C
ECM: Extracellular matrix
JAK: Janus kinase
STAT: Signal transducer and activator of transcription
ERK: Extracellular-signal-regulated kinase
MAPK: Mitogen-activated protein kinase

NAD(P)H: Nicotinamide adenine dinucleotide phosphate
NFκB: Nuclear factor kappa-light-chain-enhancer of activated B cells
AP-1: Activator protein 1
EGFR: Epidermal growth factor receptor
TGFβ: Transforming growth factor beta
PAI-1: Plasminogen activator inhibitor-1
MCP-1: Monocyte chemotactic protein-1
ACEI: ACE inhibitor
BBB: Blood-brain barrier
CB: Ciliary body
RGC: Retinal ganglion cell
BRB: Blood-retina barrier
ERG: Electroretinogram
EIU: Endotoxin-induced uveitis
LPS: Lipopolysaccharide
ICAM-1: Intercellular adhesion molecule 1
IL-6: Interleukin 6
IFN-γ: Interferon-gamma
EAU: Experimental autoimmune uveoretinitis
IRBP: Interphotoreceptor retinoid-binding protein
PRRB: (Pro)renin receptor blocker
VEGF: Vascular endothelial growth factor
VEGFR: VEGF receptor
OIR: Oxygen-induced retinopathy
AGE: Advanced glycation end-product
PDR: Proliferative diabetic retinopathy
DME: Diabetic macular edema
EUCLID: EURODIAB Controlled Trial of Lisinopril in Insulin-Dependent Diabetes
DIRECT Diabetic Retinopathy Candesartan Trial
RASS: Renin-Angiotensin System Study
STZ: Streptozotosin
GLUT-1: Glucose transporter 1
CNV: Choroidal neovascularization
MMP: Matrix metalloproteinase
IOP: Intraocular pressure
NTG: Normal tension glaucoma
GFAP: Glial fibrillary acidic protein
NMDA: N-methyl-D-aspartic acid.

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Research Article

IL-8 and MCP Gene Expression and Production by LPS-Stimulated Human Corneal Stromal Cells

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Purpose. To determine time course of effect of lipopolysaccharide (LPS) on production of interleukin-8 (IL-8) and monocyte chemotactic protein (MCP) by cultured human corneal stromal cells. *Methods*. Human corneal stromal cells were harvested from donor corneal specimens, and fourth to sixth passaged cells were used. Cell cultures were stimulated with LPS for 2, 4, 8, and 24 hours. Northern blot analysis of IL-8 and MCP gene expression and ELISA for IL-8 and MCP secretion were performed. ELISA results were analyzed for statistical significance using two-tailed Student's *t*-test. *Results*. Northern blot analysis demonstrated significantly increased IL-8 and MCP gene expression after 4 and 8 hours of exposure to LPS. ELISA for secreted IL-8 and MCP demonstrated statistically significant increases ($P < 0.05$) after corneal stromal cell stimulation with LPS. *Conclusions*. This paper suggests that human corneal stromal cells may participate in corneal inflammation by secreting potent leukocyte chemotactic and activating proteins in a time-dependent manner when exposed to LPS.

1. Introduction

Leukocytic infiltration of the cornea is a common and important pathologic process observed in infection, autoimmune diseases, allograft rejection, and surgical and nonsurgical trauma. Soluble chemotaxins, such as interleukin-8 (IL-8) and monocyte chemotactic peptide (MCP), both appear to be essential to leukocyte recruitment, accumulation, and activation at sites of inflammation. IL-8 and MCP are distinct polypeptides that directly mediate leukocyte chemotaxis *in vitro* and *in vivo* and may be secreted by tissue-based cells exposed to inflammatory cytokines [1, 2]. Both IL-8 and MCP have been shown to be produced by human corneal tissue in response to inflammatory stimuli [3, 4].

Since the cornea is normally avascular, identification of chemotaxins that elicit corneal leukocyte infiltration may be pertinent to understanding pathogenetic mechanisms regulating corneal inflammation and immunity. Lipopolysaccharide (LPS) is a component of gram-negative bacteria cell membrane that is known to induce the innate immune response. The importance of corneal leukocytic infiltration in disease has prompted prior investigations of corneal-derived chemotactic factors, including lipopolysaccharide (LPS) [5–8], but the specific time- and dose-dependent properties of IL-8 and MCP in response to LPS have not previously been identified.

In this study, we evaluated cultured human corneal stromal cells for the production of IL-8 and MCP using northern blot analysis to assess gene expression and enzyme-linked immunoabsorbent assays (ELISA) to measure corneal stromal cell secretion of IL-8 and MCP product in response to stimulation by LPS.

2. Materials and Methods

2.1. Human Corneal Stromal Cell Cultures. Human donor corneal specimens of good quality, but unsuitable for transplantation, were obtained from the Michigan Eye Bank and Transplantation Center or the Illinois Eye Bank. Within 24

hours of death, corneal stromal cells were harvested by trimming all limbal tissue, mechanically removing the epithelium and endothelial cells, and establishing the corneal stromal cells in Dulbecco's modified essential medium containing 15% fetal bovine serum (FBS). Fourth to sixth passaged cells were used for all assays. Before stimulation with cytokines, the cell cultures were rinsed with fresh, serum-free medium.

2.2. Human Corneal Stromal Cell Stimulation with Cytokines. Assayed stromal cells were either left unstimulated or treated with lipopolysaccharide (LPS; Escherichia coli 0111:B4, Sigma Chemical Co., St. Louis, MO) for 2, 4, 8, and 24 hours. Cultured cells were also exposed to LPS at specified concentrations (1, 10, 100, 1000, 10,000 ng/mL) for 8 hours. After experimental incubations, culture media were collected and stored at −70°C until ELISA assays for IL-8 or MCP were performed and the cell monolayers were extracted for IL-8 and MCP mRNA analysis.

2.3. Northern Blot Analysis of Human Corneal Stromal Cell IL-8 and MCP mRNA. Human corneal stromal cell monolayers were solubilized in 25 mM TRIS containing 4.2 M guanidine isothiocyanate, 9.5% Sarkosyl, and 0.1 M β-mercaptoethanol. Total corneal stromal cell RNA was extracted, and Northern blots prepared by extracting RNA, which was separated by electrophoresis, transferred to nitrocellulose and hybridized with either a ^{32}P-5′-end-labeled 30 nucleotide probe complementary to either nucleotides 262 to 291 of the published cDNA sequence for IL-8 or to nucleotides 256 to 285 of the published cDNA sequence for MCP. The blots were washed and autoradiographed. Equivalent amounts of RNA in each Northern blot was assessed by monitoring equivalence of 28s and 18s rRNA signals.

2.4. IL-8 and MCP ELISA Assays of Human Corneal Stromal Cell Supernatants. Immunoreactive IL-8 or MCP was measured in corneal stromal cell supernatants using a modification of a double ligand ELISA method. Briefly, 96-well microtiter plates were coated with either rabbit anti-IL-8 or anti-MCP antibody. Sequential incubations with biotinylated rabbit anti-IL-8 (1 : 2000) or anti-MCP then performed and chromogen substrate was added. The plates were incubated to the desired extinction and the reaction terminated. Plates were read in an ELISA reader and calibrated using 1/2 log dilution standards of rIL-8 or rMCP concentrations ranging from 1 pg to 1000 ng/well. Under each condition, the ELISA was performed 1 mL of media covering 500,000 cells. This ELISA method consistently detected IL-8 or MCP concentrations greater than 10 pg/mL in a linear fashion.

2.5. Statistical Analysis. Individual experiments were performed 4 times on 4 different corneal stromal cell lines. All data were expressed as means ± standard error of the means. The various assay conditions were compared using Student's t-test and P-values less than 0.05 were considered to be statistically significant.

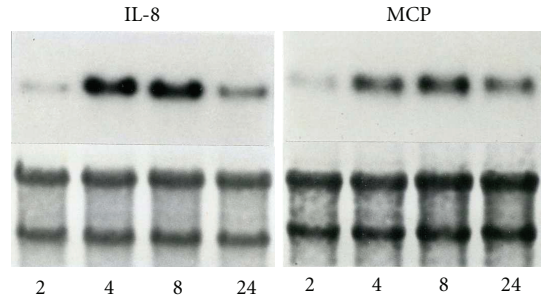

Figure 1: Time-dependent human corneal stromal cell mRNA expression of IL-8 and MCP detected by Northern blot analysis following lipopolysaccharide (LPS) stimulation of 2, 4, 8, and 24 hours.

3. Results and Discussion

Northern blot analysis was performed to assess mRNA expression of IL-8 and MCP by human corneal stromal cells in response to varying exposure times (2, 4, 8, and 24 hours) to LPS at a concentration of 1000 ng/mL. mRNA expression of both IL-8 and MCP was demonstrated to be minimal after 2 hours of exposure to LPS, but then increased substantially after 4 to 8 hours of exposure to LPS (Figure 1). mRNA expression of both IL-8 and MCP was decreased after 24 hours of LPS exposure, with a greater decrease seen in IL-8 than MCP expression (Figure 1). No induction or mock induction controls were used in this analysis.

ELISA was performed to assess the expression of IL-8 and MCP by human corneal stromal cells after exposure to LPS (1000 ng/mL) for 2, 4, 8, and 24 hours. A low level of constitutive IL-8 and MCP was consistently determined on unstimulated control cells. A substantial increase was seen relative to unstimulated control with stimulation of LPS as soon as 2 hours after exposure, and at all measured time points ($P < 0.05$) (Figures 2 and 3). Expression of both IL-8 and MCP-1 increased progressively with length of time of exposure to LPS ($P < 0.05$) (Figures 2 and 3).

To determine the effect of LPS concentration on human corneal stromal cell IL-8 and MCP expression, cells were incubated for 8 hours with different concentrations of LPS (1, 10, 100, 1,000, and 10,000 ng/mL). ELISA was used to compare the IL-8 and MCP expression at the different concentrations of LPS. Increasing concentrations of LPS led to progressive increases in IL-8 expression which were statistically significant ($P < 0.05$) (Figure 4). There was also increased MCP expression with increasing concentrations of LPS, but MCP expression reached a plateau at LPS concentrations greater than 100 ng/mL (Figure 5). MCP expression was statistically significantly increased from baseline at all concentrations greater than 1 ng/mL ($P < 0.05$).

4. Conclusions

Circulating leukocytes infiltrate tissue at sites of inflammation by binding to cellular adherence molecules expressed by tissue-based cells and migrating along gradients of specific

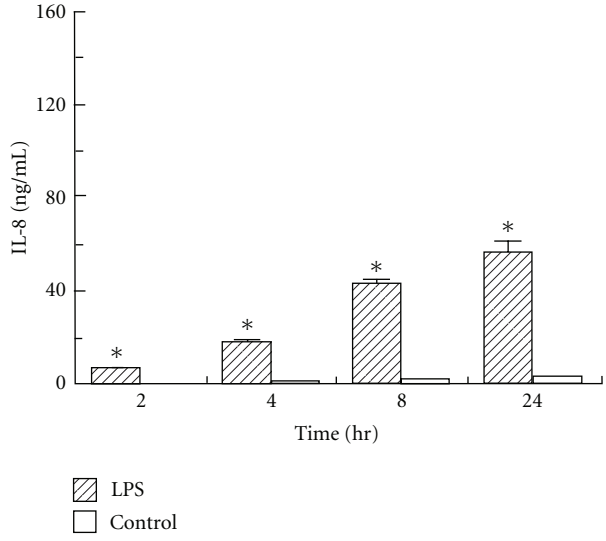

Figure 2: Time-dependent human corneal stromal cell secretion of IL-8 detected by ELISA following lipopolysaccharide (LPS) stimulation of 2, 4, 8, and 24 hours. $^*P < 0.05$ compared to unstimulated control.

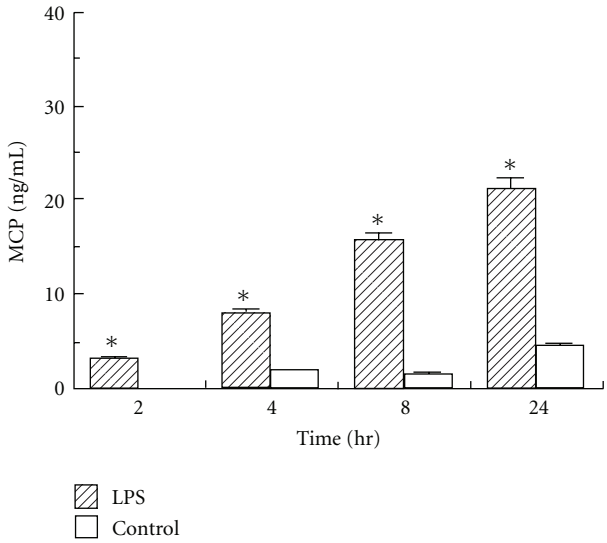

Figure 3: Time-dependent human corneal stromal cell secretion of MCP detected by ELISA following lipopolysaccharide (LPS) stimulation of 2, 4, 8, and 24 hours. $^*P < 0.05$ compared to unstimulated control.

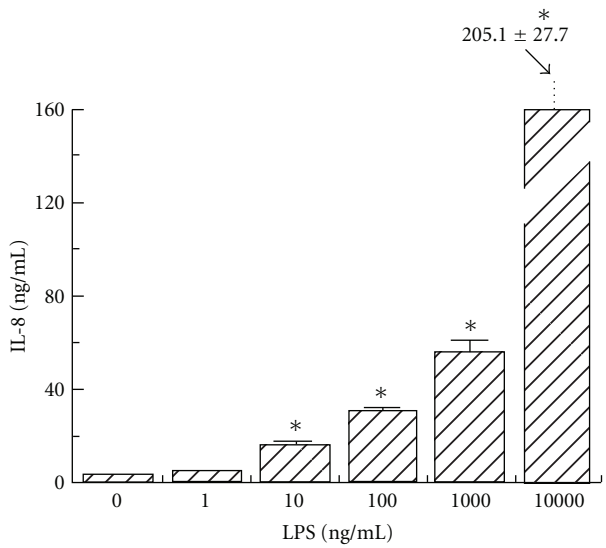

Figure 4: Dose-dependent human corneal stromal cell secretion of IL-8 detected by ELISA following lipopolysaccharide (LPS) stimulation at 1, 10, 100, 1,000, and 10,000 ng/mL concentrations. $^*P < 0.05$ compared to unstimulated control.

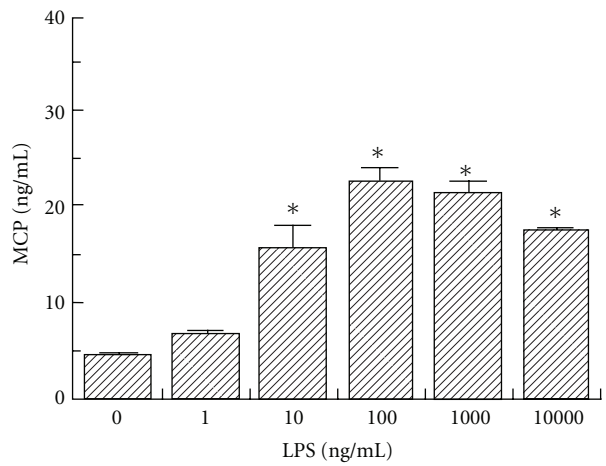

Figure 5: Dose-dependent human corneal stromal cell secretion of MCP detected by ELISA following lipopolysaccharide (LPS) stimulation at 1, 10, 100, 1,000, and 10,000 ng/mL concentrations. $^*P < 0.05$ compared to unstimulated control.

chemotactic factors. Although the precise stimuli initiating inflammation vary, they appear to trigger common pathogenetic cascades leading to leukocyte elicitation and activation. LPS is an important mediator of inflammation that has been shown to induce de novo synthesis of specific leukocyte chemotaxins, such as IL-8 and MCP, by tissue-based cells.

Our results show that IL-8 and MCP are chemotaxins that are significantly induced in corneal stromal cells by LPS stimulation. Our findings suggest that LPS stimulation of steady-state corneal stromal cell IL-8 and MCP mRNA is prompt and is subsequently followed by secretion of significant levels of these cytokines human corneal stromal cells.

This induction is time dependent, with exposures of 4 to 8 hours inducing the highest increase in mRNA expression seen in our assays. There is then a decrease in mRNA expression at 24 hours, possibly indicating a saturation effect of this pathway of acute inflammation. This decrease was more marked with IL-8 mRNA expression than MCP, likely associated with the role of MCP in more chronic inflammation.

Our results show that both IL-8 and MCP protein expression continues to increase over the first 24 hours of exposure

to LPS. It would be interesting to proceed with a longer-term study to evaluate the time dependency of the protein expression of these chemokines. Our study also evaluates the effect of concentration of LPS on protein expression of IL-8 and MCP by corneal stromal cells. IL-8 expression increased dramatically in response to increasing concentrations of LPS. On the other hand, MCP expression reached a maximum at an LPS concentration of 100 ng/mL, and then seemed to plateau or even show slight decreases in expression at higher concentrations of LPS. This unusual dose response at higher concentrations requires further study.

Previous studies have shown that LPS from several different bacterial species induces increased IL-8 and MCP mRNA by quantitative RT-PCR and increased protein expression by ELISA [5]. It has also been shown that increased IL-8 and MCP mRNA and protein expression in corneal fibroblasts is potentiated by LPS-binding protein and soluble CD14 [6]. To our knowledge, this is the first study to show the time-course and dose dependence of the induction of IL-8 and MCP in human corneal stromal cells after exposure to LPS.

Once secreted, corneal-derived IL-8 and MCP may elicit their inflammatory effects by binding specific receptors on neutrophils, lymphocytes, and monocytes, leading to leukocyte accumulation and activation at the site of inflammation [3, 4]. The elaboration of IL-8 and MCP by tissue-based cells may be an especially important mechanism directing leukocytes to migrate over long distances through tissue in which blood vessels containing circulating leukocytes are absent, such as the normally avascular cornea. The induction of IL-8 and MCP is one of many events that occur in response to LPS. Thus, corneal stromal keratocytes may produce soluble, diffusible chemotaxins, including IL-8 and MCP, that may be important in the inflammatory cascade response to LPS stimulation.

Acknowledgments

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Research Article

Resident Corneal Cells Communicate with Neutrophils Leading to the Production of IP-10 during the Primary Inflammatory Response to HSV-1 Infection

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In this study we show that murine and human neutrophils are capable of secreting IP-10 in response to communication from the HSV-1 infected cornea and that they do so in a time frame associated with the recruitment of $CD8^+$ T cells and CXCR3-expressing cells. Cellular markers were used to establish that neutrophil influx corresponded in time to peak IP-10 production, and cellular depletion confirmed neutrophils to be a significant source of IP-10 during HSV-1 corneal infection in mice. A novel *ex vivo* model for human corneal tissue infection with HSV-1 was used to confirm that cells resident in the cornea are also capable of stimulating neutrophils to secrete IP-10. Our results support the hypothesis that neutrophils play a key role in T-cell recruitment and control of viral replication during HSV-1 corneal infection through the production of the T-cell recruiting chemokine IP-10.

1. Introduction

Herpes simplex virus-type 1 (HSV-1) infection of the human cornea can lead to a damaging inflammatory response known as herpes stromal keratitis (HSK). According to the National Eye Institute, 50,000 new and recurring HSV-1 ocular infections are reported annually, and HSK is the leading cause of infectious blindness in the United States [1]. During physical trauma or HSV-1 infection, cells resident in the cornea initiate an immune response through the production of proinflammatory mediators such as IL-1α, IL-6, and the neutrophil chemoattractant CXCL8 (homologous to MIP-2 in the mouse) [2–5]. Mice also exhibit an inflammatory response to HSV-1 infection of the cornea which is marked by neutrophil infiltration at days 2 and 9 after infection (p.i.) [6–9]. In the mouse, $CD8^+$ T cells are required for viral clearance around day 8 after infection, while the $CD4^+$ T-cell subset has been described as having a role in the development of HSK and $CD8^+$ cell regulation after viral clearance has occurred [8, 10, 11].

The role of the neutrophil in the immune response to HSV-1 infection has not yet been fully explored. However, neutrophil depletion studies have demonstrated that in the absence of neutrophils $CD8^+$, T-cell levels are reduced and viral clearance is limited leading to more severe disease development [12, 13]. Divito and Hendricks [14] reported that neutrophil accumulation in addition to $CD4^+$ T-cell infiltration was necessary for HSK development. Previous studies reported from our lab and others indicate a role for neutrophils in the secretion of T-cell-recruiting chemokines IP-10 (Interferon gamma-induced protein 10 or CXCL10) and MIG (Monokine induced by gamma interferon or CXCL9) [15, 16]. It is known that T lymphocytes express the receptor CXCR3 and may be recruited by the ligands IP-10 and MIG and that antibody neutralization of IP-10 results in increased viral titers during HSV-1 corneal infection [17, 18]. We previously used a model for delayed type hypersensitivity (DTH), a secondary immune response to HSV-1 antigen in the skin of mice, to demonstrate that the neutrophil acts as a source for both IP-10 and MIG in the model. In the absence

of neutrophil recruitment, T lymphocyte numbers were reduced during the DTH response [15, 19]. Thus, although the neutrophil has traditionally been described as merely a phagocytic cell, there is now increasing evidence to support the hypothesis that it may also function to bridge the response between the innate and adaptive immune system.

In the study presented here, we hypothesize that neutrophils play a role in T-cell recruitment into the HSV-1 infected cornea through the production of IP-10. We describe the use of a model for the inflammatory response to a primary HSV-1 infection of the murine cornea and a novel *ex vivo* system for the study of primary HSV-1 infection in human corneas. Data is presented from experiments designed to investigate the production kinetics of chemokine IP-10 and its receptors during infection. We also demonstrate the effect of cellular depletion of neutrophils, natural killer cells, and $CD4^+$ T cells on the level of IP-10 production and provide evidence for secretion of IP-10 by neutrophils, *in vitro* and *in vivo*, in both murine and human corneal tissue infected by HSV-1.

2. Materials and Methods

2.1. Mice. Six-week-old female C57Bl/6 mice were obtained from Jackson Laboratories (Bar Harbour, ME). All animals were cared for in accordance with federal, state, and local regulations.

2.2. Antibodies and Reagents. Anti-mouse granulocyte mAb RB6-8C5 was used for the depletion of neutrophils and was a gift from Robert Coffman (DNAX Research Institute, Palo Alto, CA). Anti-mouse CD4 hybridoma clone GK1.5 was obtained from the American Type Culture Collection (ATCC, Manassas, VA). Antibodies were prepared from hybridoma lines as previously described [13]. A combination of rabbit antiasialo GM1 (Wako Pure Chemical Industries, Ltd., Richmond, VA) and mAb NK1.1 (BD Biosciences, San Diego, CA) were used for NK cell depletion. Recombinant mouse IL-1α and IFN-γ) were purchased from R&D Systems (Minneapolis, MN.).

2.3. Topical and Intracorneal HSV-1 Infection of Murine Corneas. For experiments investigating the protein kinetics of IP-10, infections were achieved topically by applying 2×10^5 PFU/2 μL HSV-1 strain RE on the scarified cornea of the mice. For mRNA investigation and cellular depletion studies, infection was performed using an intrastromal route to reduce intragroup variability. In brief, a pilot hole was formed through the epithelium of the cornea, with a 30-gauge disposable needle, through which a 32-gauge, 30 cm needle attached to a repeating dispenser (Hamilton, Reno, NV) was then inserted. 1×10^4 or 1×10^5 PFU HSV-1 RE (as indicated in the figure legend) was injected intrastromally in a volume of 1 μL.

2.4. Corneal Opacity Scores. Eyes were monitored for corneal opacity by visual observation under the dissecting microscope and graded as follows: 0 = clear cornea, 1 = slight corneal haze, 2 = moderate corneal opacity, 3 = severe corneal opacity but iris visible, 4 = severe corneal opacity with iris obscured, and 5 = necrotizing stromal keratitis.

2.5. Virus Titration. Corneas were titrated for infectious virus on vero cell monolayers in a standard 48 h plaque assay.

2.6. mRNA Extraction and Real-Time PCR Analysis. In experiments where upregulation of mRNA levels was investigated, corneas from 5 mice of the same experimental group were excised and pooled. The pooled corneas were homogenized for 30 s in 0.5 mL of RNAwiz (Ambion Inc. Austin, TX) and total RNA was then extracted following the manufacturers protocol. Contaminating genomic DNA was removed by DNase treatment using DNA free (Ambion Inc., Austin, TX), while total RNA purity and quantity were determined using the Bio-Rad SmartSpec 3000 spectrophotometer (Hercules, CA). Ambion Message Sensor RT (reverse transcriptase) kit was used to convert 0.5 μg total RNA to cDNA for real-time PCR analysis. Bio-Rad iQ SYBR Green Supermix in a 96-well plate format was used for analysis with a Bio-Rad iCycler IQ system. GAPD mRNA levels were used to normalize template loading variations and negative RT reactions were performed to ensure the absence of genomic contamination in the samples. mRNA levels are reported as a fold increase over expression levels in untreated control corneas.

2.7. Murine Corneal Fibroblast Cell Culture. Sixteen corneas from 8 uninfected mice were excised, pooled, and incubated at 37°C, 5% CO_2 in 5 mM EDTA/PBS for 20 min. After this time, epithelial sheets were removed with forceps under the dissecting microscope. The stromal layers of each cornea were minced and incubated in 1500 U/mL of collagenase type I (Sigma) at 37°C, 5% CO_2, for 1 h. The digested stromal layers were washed repeatedly in DMEM containing 20% FBS. Resultant cells were cultured in a T25 tissue culture flask in minimal volume of DMEM + 20% FBS medium. After 1 week of culture, cells were trypsinized and redistributed to the same flask to avoid clumping. At 90% confluency cells were passaged to a 12-well tissue culture plate at a density of 4×10^4 cells/well. Cells were serum starved for 3 days prior to stimulation for 24 h with IFN-γ or IL-1α. Each stimulation was performed in triplicate and chemokine production was quantitated by ELISA.

2.8. Murine Neutrophil Isolation and Stimulation. Neutrophils were isolated from the bone marrow of mice as previously described [20]. In brief, bone marrow cells extracted from the hind limbs of mice were gradient purified over Histopaque 1119 and 1077 (Sigma, St. Louis, MO). The enriched neutrophil band was washed in medium and treated with red blood cell lysis buffer (Sigma). Contaminating monocytes were depleted by adherence to a polystyrene culture plate. Neutrophil purity was established to be consistently >99%, by HEMA3 (Biochemical Sciences) staining of cytospin slides. For *in vitro* stimulation assays, 1×10^6 neutrophils in 0.5 mL medium were placed in triplicate in

24-well tissue culture plates (Corning, New York, NY) previously coated with newborn calf serum (NCS). Neutrophils were stimulated for 8 h at 37°C, 5% CO_2, with IL-1α, IFN-γ, or HSV-1. Medium alone was used as a negative control. Supernatants were collected, clarified, and assayed by ELISA for protein levels.

2.9. Chemokine Protein Assay. Corneas were excised and cleaned of limbal tissue at the indicated times and processed by homogenization (30 seconds in a tissue tearer; Biospec Products, Bartlesville, OK) and sonication (15 s) in a total of 0.5 mL RPMI + 10% NCS unless stated otherwise. Corneal samples were clarified by centrifugation at 150 × g for 10 min and supernatants were analyzed by ELISA. In cell studies, supernatants were collected and assayed for secreted chemokine levels by ELISA. Murine IP-10 and MIP-2 ELISA assay kit sensitivities were 2.2 and 1.2 pg/mL, respectively, and the human IP-10 assay kit had a mean minimal detectable dose of 1.67 pg/mL. All kits were obtained from R&D systems (Minneapolis, MN.).

2.10. In Vivo Cellular Depletions. To achieve *in vivo* depletion of cellular subsets, 0.5 mg of mAb RB6-8C5 (neutrophil depletion), 0.5 mg GK1.5 ($CD4^+$ T-cell depletion), or 1 mg antiasialo GMI admixed with 0.1 mg NK1.1 (NK cell depletion) were administered by intraperitoneal injection to mice 3 h prior to HSV-1 challenge [13, 21–23]. Mice were challenged by intrastromal injection of 1×10^5 PFU of HSV-1 and corneas excised at the times indicated. Depletion of neutrophils was confirmed by differential staining of blood smears. FACS analysis of spleen cells was performed using GK1.5 and NK1.1 to quantify $CD4^+$ T-cell and NK-cell depletion levels, respectively. Excised corneas were processed by homogenization and sonication to produce a lysate for chemokine analysis by quantitative ELISA.

2.11. Human Corneal Tissue Infection. Human corneas were obtained from the Georgia Eye Bank, Inc. EBAA (Atlanta, GA). All donors were screened and found to be nonreactive for HIV and Hepatitis. Corneas were released for research upon failure to meet transplantation criteria.

Four, 4 mm corneal buttons were punched from each cornea using an arch punch (C.S. Osborne Tools). A minimum of three donors were used per experiment. Corneal buttons were placed in 24-well tissue culture plates containing 0.5 mL serum free RPMI 1640 media for incubation at 37°C, 5% CO_2. Corneal button surfaces were scarified using an 18-gauge needle to mimic the murine topical infection protocol. For HSV-1 infected samples 1×10^6 PFU of virus was added to the corneal button in the well. Infected and uninfected corneas were then incubated in the presence or absence of purified human neutrophils, 1×10^6 neutrophils/well at 37°C, 5% CO_2, for 24 h. Media, cells, and corneal buttons were removed and processed by sonication (30 s). Quantitation of human IP-10 chemokine levels was performed by ELISA. Levels of the chemokine CXCL8 were monitored as a marker of inflammation. This model permits the study of the interaction between resident corneal cells, neutrophils, and virus in the absence of other cell types which could be recruited to the site of inflammation *in vivo*.

2.12. Human Neutrophil Isolation. Neutrophils were obtained from freshly donated venous blood. Gradient purification was achieved following a protocol equivalent to that described above for the murine neutrophils.

2.13. Statistical Analysis. Student's t test was performed to determine significant differences between experimental and control groups which each contained a minimum of three mice or three human corneal donors. A value of $P < 0.05$ was considered significant. A nonparametric test was performed on clinical samples where indicated in the figure legend. A representative experiment is shown in each figure with experiments having been performed multiple times.

3. Results

3.1. CXCR3 mRNA Is Detected in the Infected Murine Cornea at 6 Days Post Infection. It has been shown previously in the murine model for HSV-1 corneal infection that the virus is cleared within 8 days due to the recruitment of $CD8^+$ T lymphocytes [24]. With the focus of this study being the T-cell recruiting chemokine IP-10, it was necessary to establish that the T lymphocytes recruited to the HSV-1 infected cornea were expressing the CXCR3 receptor for IP-10. Mice were infected by intrastromal injection of 1×10^4 PFU HSV-1 and corneas harvested 2–6 days post infection (p.i.) for mRNA analysis. In Figure 1, CXCR2 was determined to be upregulated >240-fold above levels found in the controls at day 2 p.i. Upregulation of CXCR2 expression was significantly reduced by day 4 and 6 (84- and 21-fold, resp.). CXCR3 mRNA was observed to be most significantly upregulated at day 6 p.i with an expression level >28-fold higher than that of the uninfected control. This coincides with a >39-fold upregulation in the expression of the CD8 marker on infiltrating cells (data not shown). These results indicate that a marker for neutrophil recruitment (CXCR2) peaks in the cornea at day 2 p.i. with HSV-1 and that CXCR3 is present in the infected cornea at a time point associated with the recruitment of $CD8^+$ T cells.

3.2. Expression of IP-10 mRNA Is Upregulated during the First 48 Hours of HSV-1 Infection of the Murine Cornea. Having demonstrated the time points for expression of a neutrophil receptor and T-cell receptor in the model, experiments proceeded to investigate the potential to produce IP-10 in a time frame appropriate for neutrophils to be the source for this T-cell-recruiting chemokine. Figure 2 shows that levels for upregulation of the mRNA for MIP-2 were found to be elevated >724-fold at day 2 p.i. dropping to >93-fold at day 4 p.i. Expression levels for IP-10 mRNA were highly upregulated (1179-fold over control) at day 2 after infection but the level of upregulation was reduced significantly by day 4 after infection (decreased to 24-fold over control). Thus, IP-10 message was found at the same time point as the peak in CXCR2 and MIP-2 mRNA expression which marked

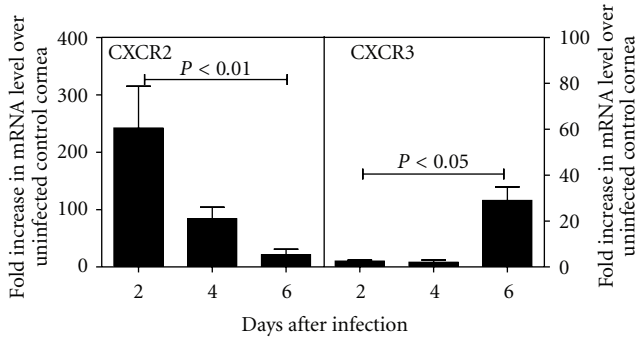

Figure 1: CXCR2 and CXCR3 mRNA are detected in the infected cornea at 2 and 6 days post infection respectively. Mice ($n = 3$) were challenged by intrastromal injection with 1×10^4 PFU HSV-1 and corneas were harvested on the days indicated post infection. Control mice were uninfected and corneas from this group represented the level of constitutive mRNA production for each receptor. Total RNA was isolated from each group and converted to cDNA which was analyzed by real-time PCR. Upregulation in mRNA for each receptor is reported as the fold increase over production in uninfected control corneas.

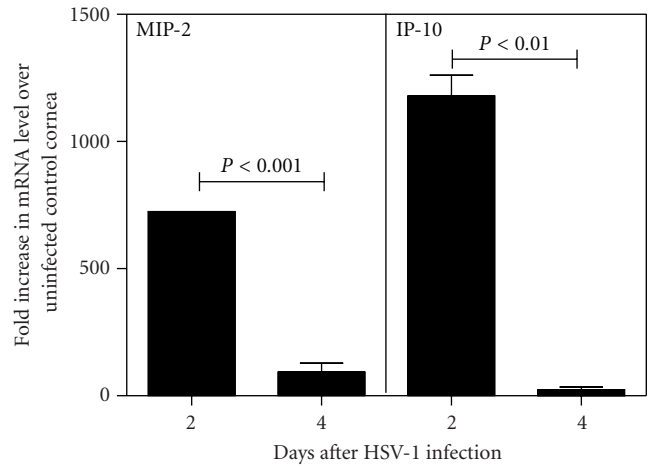

Figure 2: Expression of IP-10 mRNA is upregulated during the first 48 hours of HSV-1 infection of the cornea. Mice ($n = 3$) were challenged by intrastromal injection with 1×10^4 PFU HSV-1 and corneas were harvested at the indicated times post infection. Control mice were uninfected and corneas from this group represented the level of constitutive mRNA production for each chemokine. Total RNA was isolated from each group and converted to cDNA which was analyzed by real-time PCR. Upregulation in mRNA for each chemokine is reported as the fold increase over production in uninfected control corneas.

the infiltration of neutrophils. In addition, IP-10 mRNA expression was observed to be elevated prior to the time point for infiltration of T cells as suggested by the presence of CXCR3 mRNA (day 6 p.i.) and CD8a and CD8b mRNA (data not shown).

3.3. IP-10 is Produced at Significant Levels in the HSV-1 Infected Murine Cornea. Although chemokine and receptor mRNA expression profiles provided supporting evidence for the hypothesis that neutrophils may act as a cellular source for the T-cell-recruiting chemokine IP-10, it was still necessary to demonstrate the presence of the IP-10 protein within the model. The kinetics of IP-10 production were determined over an 8 day period after topical infection of corneas with 2×10^5 PFU HSV-1. At each time point indicated on Figure 3, mice were monitored for corneal opacity as an indicator of the level of inflammation (Figure 3(a), ■). Mice were then sacrificed and corneal lysates produced to determine virus titers by plaque assay (Figure 3(a), ∘). IP-10 protein levels were quantitated by ELISA and are shown in Figure 3(b).

Figure 3(a) demonstrates that viral load (■) was significantly reduced by day 8 p.i. compared to the day 0 inoculum and that corneal opacity due to cellular infiltration was significantly increased (∘) in the same time frame. Constitutive expression of IP-10 was observed to be >38.6 pg/mL ± 2.8 SEM at day 0 in the model. The kinetics of IP-10 production are marked by a significant peak of 861.8 pg/mL ± 136.2 SEM at day 2 after infection IP-10 levels remain significantly high until day 7 and return to constitutive levels at day 8 p.i. (130.4 pg/mL ± 70.2 SEM and 30.1 pg/mL ± 7.6 SEM, resp.). This day 2 p.i. time point for peak IP-10 protein production agrees with that observed for peak IP-10 mRNA and peak CXCR2 mRNA expression.

3.4. Cells Resident in the Murine Cornea Have the Potential to Produce IP-10. Murine corneas have been reported to be productive for mRNA for various chemokines including MIP-2 and IP-10 in a Balb/c HSV-1 infection model [25, 26]. As reported by Lundberg et al. the effects of IP-10 signaling may be strain specific [27]. Having established that IP-10 protein is produced at high levels within the C57 Bl/6 HSV-1 infected cornea, experiments were initiated to determine the potential of resident corneal cells from this strain of mouse to act as a source of IP-10. Mouse corneas were excised from uninfected hosts and incubated *ex vivo* for 24 h. Uninfected corneas were devoid of lymphocytes normally recruited during the inflammatory response to HSV-1. Constitutive levels of IP-10 protein were measured from samples which were excised and processed immediately. In Figure 4, it can be observed that constitutive levels of IP-10 were low (38.6 pg/mL ± 2.8 SEM) but that incubation for 24 hours led to a >5-fold increase in IP-10 levels. This data demonstrates that resident cells of the cornea have the potential to secrete IP-10 protein in response to excision trauma and may, therefore, contribute to the production of the chemokine during virus infection in the C57 Bl/6 model.

3.5. Cultured Murine Fibroblast Cells Produce High Levels of IP-10 in Response to Stimulation with Proinflammatory Mediators. Tissue culture experiments were performed to further explore the potential for production of IP-10 by resident corneal cells as opposed to infiltrating leukocytes. Corneas from uninfected mice were excised and processed, as described in the materials and methods, to establish a primary corneal fibroblast cell line. 4×10^4 fibroblast cells were

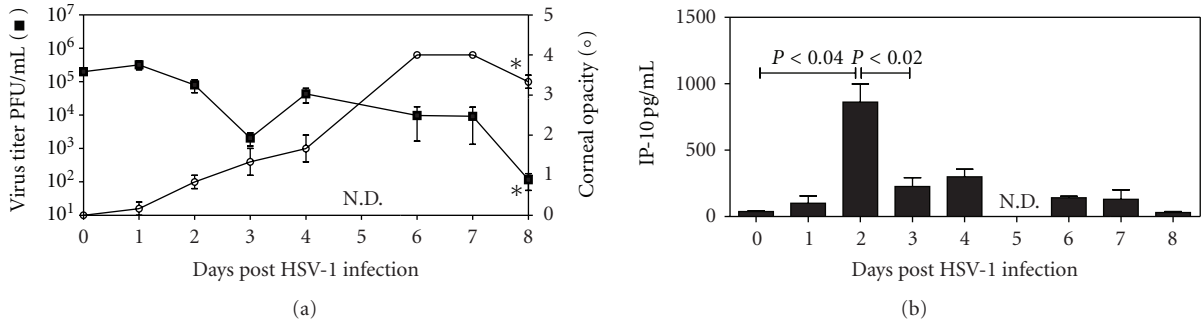

Figure 3: IP-10 is produced at significant levels in the HSV-1 infected cornea. Mice were infected with 2×10^5 PFU HSV-1 on the scarified cornea. Mice were monitored for corneal opacity (graph (a), open circle, ○) an asterisk indicates a significant increase between day 1 and 8. At the indicated time points corneas ($n = 3$) were excised and lysates produced for virus titration (graph (a), closed box, ■), an asterisk indicates a significant reduction from day 1 to day 8 (t-test) and the medians for the data are found to be significantly different by a nonparametric test (Kruskal-Wallis test). Levels of IP-10 protein production within the infected corneal lysates are shown in (b). N.D. = not done.

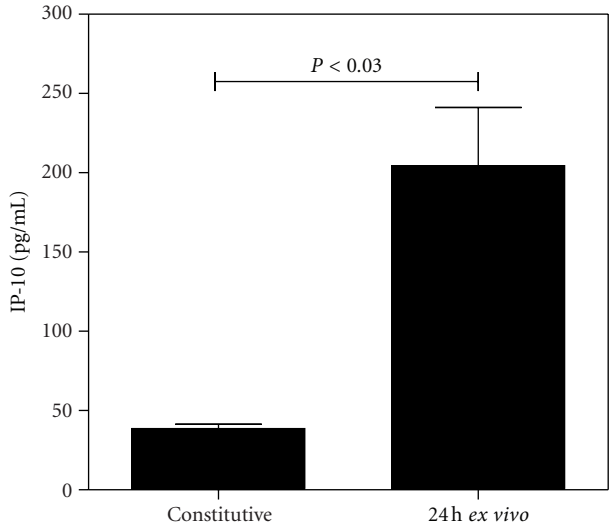

Figure 4: Cells resident in the cornea have the potential to produce IP-10. Corneas ($n = 4$) were excised from uninfected mice and placed in 250 μL of serum-free medium. Corneas were either processed immediately for analysis of chemokine production (constitutive levels) or incubated at 37°C in 5% CO_2 for 24 h. Chemokine levels were quantitated by ELISA and reported as pg/mL of sample.

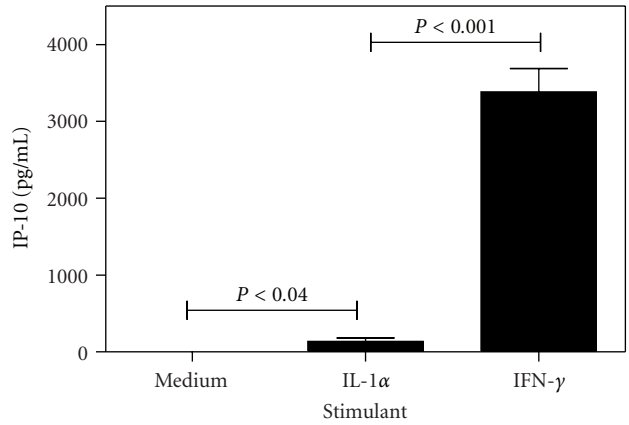

Figure 5: Cultured fibroblast cells produce IP-10 in response to stimulation with proinflammatory mediators. 4×10^4 cultured mouse corneal fibroblast cells were incubated at 37°C and 5% CO_2 in 500 μL serum-free media with or without cytokine stimulation for 24 h. Cells were stimulated with 10 ng/mL of either recombinant mouse IL-α or IFN-γ and assayed for IP-10 production by ELISA.

plated under serum-starved conditions and stimulated with the proinflammatory cytokines IL-1α or IFN-γ at 10 ng/mL for 24 h. The cell supernatants were assayed and levels of IP-10 production are shown in Figure 5. Within the time frame of the stimulation, expansion of the cell population was not observed to occur (data not shown). Fibroblast cells produced high levels of IP-10 after stimulation with IFN-γ (3383 pg/mL ± 306 SEM). Although IL-1α stimulation also lead to significant IP-10 production compared to the media control, the level was >24-fold lower than that observed for IFN-γ stimulation. The ability of murine corneal fibroblast cells to produce IP-10 in response to stimulation with proinflammatory mediators was confirmed.

3.6. Purified Murine Neutrophils Produce High Levels of IP-10 in Response to Stimulation with Proinflammatory Mediators or HSV-1. Neutrophils are rapidly recruited to the HSV-1 infected cornea and are present in high numbers at 2 day after infection [4]. We observe this to also be the time point for peak IP-10 production in the model. We performed *in vitro* stimulation of bone marrow-derived neutrophils with IFN-γ, IL-1α or HSV-1 in order to determine the neutrophils potential to secrete IP-10. Figure 6 illustrates that neutrophils produce high levels of IP-10 (4068 pg/mL ± 50 SEM) after incubation with 10 ng/mL IFN-γ for 8 h but did not produce significant levels of IP-10 after stimulation with the same concentrations of IL-1α. Neutrophils also responded to the presence of HSV-1 at a multiplicity of infection (M.O.I) of 0.1 by producing 937 pg/mL ± 126.5 SEM IP-10. A 10-fold increase in number of virus particles led to a 3.9-fold increase in chemokine production. Thus, neutrophils were

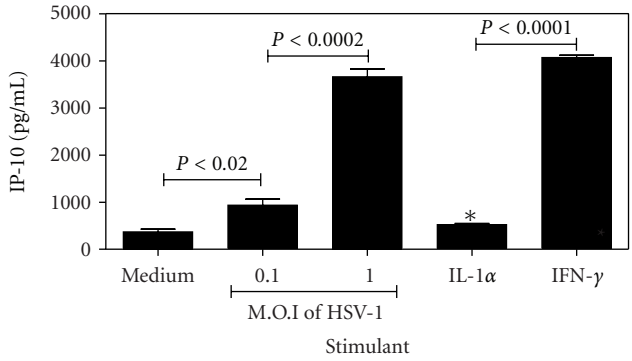

Figure 6: Purified mouse neutrophils produce high levels of IP-10 in response to stimulation with proinflammatory mediators or HSV-1. 1×10^6 neutrophils purified from bone marrow were stimulated for 8 h *in vitro* with 10 ng/mL of either recombinant mouse IL-1α, IFN-γ, or HSV-1 at the dose indicated. Chemokine production was assayed by ELISA. An asterisk indicates a value not significantly different from the unstimulated control.

demonstrated to have the ability to produce IP-10 in response to both proinflammatory cytokines and the presence of HSV-1. These results indicate a potential for neutrophils to contribute to the production of IP-10 within the HSV-1 infected cornea.

3.7. Neutrophil or NK Cell Depletion Lead to Significant Reduction of IP-10 Levels in the HSV-1 Infected Murine Cornea. Having established that neutrophils were a potential cellular source of IP-10 *in vitro,* we performed *in vivo* depletion of these cells to determine their contribution to chemokine production during a primary HSV-1 corneal infection. NK and $CD4^+$ T cells infiltrate the HSV-1-infected cornea [9] and have the potential to secrete cytokines including IFN-γ which could in turn stimulate neutrophils to secrete IP-10. We therefore included depletion of NK and $CD4^+$ cells in this investigation. Peripheral neutrophil depletion was assessed by differential staining of tail vein blood smears to be reduced by >84% of the cell number observed in the IgG-control-treated mice. $CD4^+$ T-cell and NK-cell depletions were monitored by FACS analysis of spleen cells and cell numbers determined to be reduced by >89% and >67%, respectively, when compared to the IgG control.

Two and three days post infection, corneas were harvested and analyzed to determine the effect of cellular depletion on IP-10 production. Figure 7 shows a representative experiment. Neutrophil depletion by antibody treatment with RB6 8C5 produced a significant 2-fold reduction in IP-10 protein levels when compared to the IgG group on day 2 p.i. By day 3 p.i. IP-10 levels in the control corneas were greatly reduced (299 pg/mL ± 103 SEM) when compared to those observed at day 2 p.i. (931.4 pg/mL ± 111 SEM), and the effect of neutrophil depletion was abrogated. NK depletion also led to a 2-fold reduction in IP-10 levels at day 2 p.i. compared to the control. At day 3 p.i. the levels of IP-10 in the NK cell depletion group were not significantly different from that of the control. $CD4^+$ T-cell depletion was not conducted at day 2 p.i. and does not significantly affect IP-10 production at day 3 p.i. in the model. These results indicate a role for both neutrophils and natural killer cells in the production of IP-10 in the model.

3.8. Corneal Cells Communicate with Neutrophils Leading to the Production of IP-10 during Ex Vivo Incubation of Human Corneas. In order to relate the findings of the murine model to infection of human corneal tissue with HSV-1, a novel *ex vivo* model was designed using donated human corneas. As described in the material and methods, this model permits the interaction of resident corneal cells, HSV-1, and neutrophils to be studied in the absence of other recruited inflammatory leukocytes which may be present *in vivo*. Corneal tissue for each experiment was obtained from a minimum of three independent donors and sectioned into 4 mm buttons. Results are shown in Figure 8. Corneal buttons from a single cornea were placed in one of the 4 test groups: control incubation with media (media column), incubation with 1×10^6 neutrophils (PMN column), infection with 1×10^6 PFU HSV-1, a M.O.I. = 1 with respects to the PMN (HSV-1 column) or infection with HSV-1-plus incubation with neutrophils (PMN + HSV-1 column). Corneal buttons incubated in isolation produced 33.7 pg/mL IP-10 ± 13.3 SEM. Infection of corneal buttons with HSV-1 failed to significantly increase IP-10 levels above this control. Incubation of corneal buttons in the presence of neutrophils led to a 3-fold increase in the production of IP-10 (96.8 pg/mL ± 21.9 SEM). HSV-1-infected corneas incubated in the presence of neutrophils also produced significant levels of IP-10 (100.4 pg/mL ± 25.7 SEM). Neutrophils incubated in isolation under conditions equivalent to those of the corneal tests produced relatively low levels of IP-10 which were significantly elevated on addition of HSV-1. Levels of CXCL8 production were also monitored for each corneal button and found to be significantly higher in HSV-1-infected corneas compared to the uninfected control (data not shown). CXCL8 is a proinflammatory chemokine produced at high levels during HSV-1 infection of the murine and human cornea. It is responsible for the recruitment of the neutrophil to the site of infection and marks the development of an inflammatory response [4].

Corneal infection with HSV-1 in the absence of neutrophils was thus determined to be insufficient for elevated production of IP-10 in this model. Levels of IP-10 were observed to be elevated when traumatized or HSV-1-infected corneas were incubated with neutrophils. These results indicate that cellular interaction between corneal cells and neutrophils contribute to IP-10 production in a novel *ex vivo* human model and are supportive of the findings described above for the mouse model.

4. Discussion

It is known that both neutrophils and $CD8^+$ T cells are key players in the inflammatory response to primary HSV-1 infection of the cornea and that $CD8^+$ T cells are required for viral clearance [12, 13, 24]. Although it is reported that

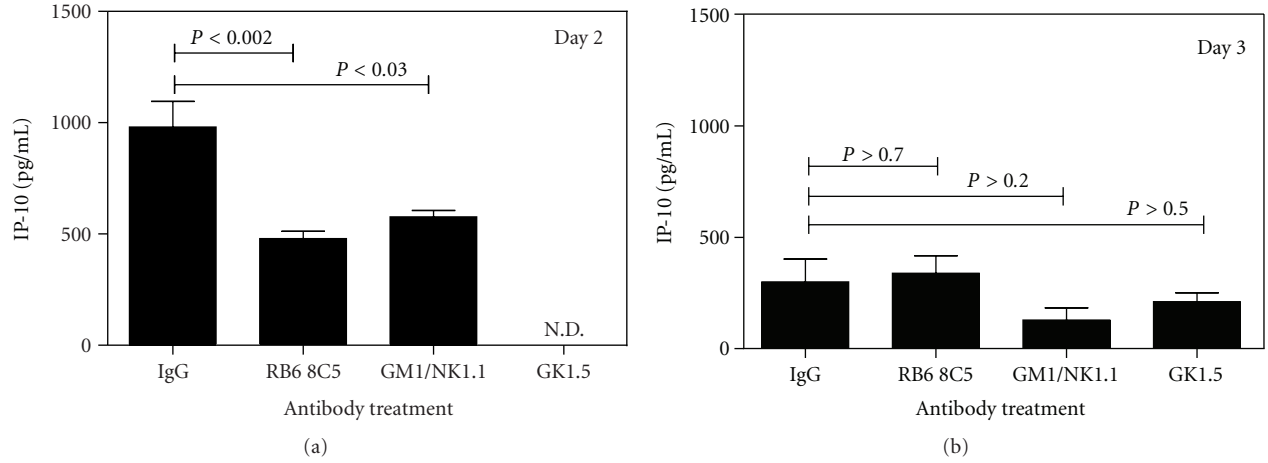

Figure 7: Neutrophil and NK depletion lead to a significant reduction of IP-10 in the HSV-1-infected cornea. Different groups of mice ($n = 4$) were depleted of each subset of cells by intraperitoneal injection 1 mg mAb RB6 8C5 (neutrophils), 1 mg antiasialo GM1 antibody admixed with 0.1 mg mAb, NK1.1 (NK cells) or 0.5 mg GK1.5 antibody ($CD4^+$ cells) three hours prior to intrastromal injection of 1×10^5 PFU HSV-1. Control animals received intraperitoneal injection of rat IgG prior to virus infection at the same dose. At days 2 and 3 post infection corneas were collected and processed for chemokine analysis via ELISA. Levels of IP-10 protein production within the infected cornea are shown as pg/mL for each sample. Cellular depletions were confirmed as described in the materials and methods. N.D. = not done.

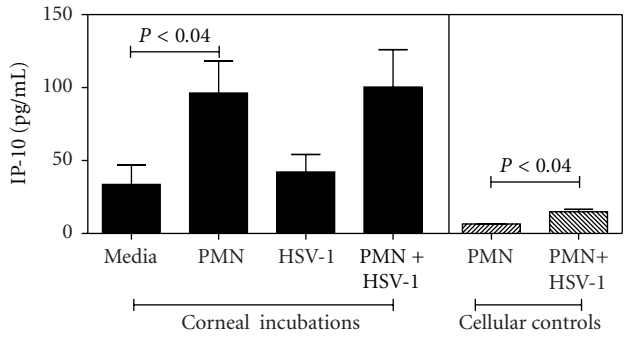

Figure 8: Corneal cells communicate with neutrophils leading to the production of IP-10 during *ex vivo* incubation of human corneas. 4 mm corneal buttons from human donor tissue were incubated in 500 μL serum-free media at 37°C, 5% CO_2 for 24 h. Where indicated corneal buttons were incubated in the presence of 1×10^6 neutrophils and/or HSV-1 at a M.O.I. = 1. Cell controls were included to monitor IP-10 production in the absence of corneal tissue. IP-10 levels are shown as pg/mL of sample.

neutrophils are also involved in the process of viral clearance, their specific mode of action is not yet fully understood [13]. Work by Gasperini et al. has previously demonstrated that human neutrophils can be stimulated to produce IP-10 and that neutrophil secretions are potent chemoattractants for NK and Th1 cells [28]. The requirement for IP-10 and CXCR3 during T-cell recruitment and activation in various inflammatory models has been established with the use of CXCR3 knockout mice. A model for *Bordetella* respiratory infection reported IP-10 induction and CXCR3 expression early during the inflammatory response and reported reduced lymphocyte and NK recruitment when CXCR3 knockout mice were investigated [29]. The requirement for CXCR3 signaling for mobilization and activation of NK and $CD8^+$ T cells and the requirement for IP-10 for viral clearance during HSV-1 and HSV-2 infections has also been described [30–33]. In addition, neutrophils have been identified as a cellular source for IP-10 during ulcerative colitis disease and are required to sustain $CD8^+$ cell recruitment during acute myocarditis [34, 35]. We have previously published data which indicates that neutrophils contribute to production of IP-10 and recruitment of $CD4^+$ T cells in an inflammatory model of delayed type hypersensitivity (DTH) to HSV-1 in the mouse [19]. We now present data which confirms IP-10 is produced in a model for corneal inflammation due to HSV-1 infection and that neutrophils are a source of this chemokine *in vivo*.

To generate further evidence linking the involvement of neutrophils in T-cell recruitment to the HSV-1-infected cornea, a time line for cellular marker and chemokine expression was generated. Due to the technical difficulties associated with the isolation and quantitation of cells infiltrating the cornea, we chose to use a semiquantitative real-time PCR screening assay to monitor receptor expression within the murine model. We established that the assay was predictive for cellular infiltration by confirming that CD3 and CD8 mRNA (T cell markers) were significantly upregulated in corneas at 6 days p.i. (data not shown). Continued screening demonstrated upregulation of mRNA for both the chemokine IP-10 and its receptor, CXCR3, at day 2 and 6 p.i., respectively. Day 2 p.i. was further marked by upregulation of the MIP-2 chemokine receptor (CXCR2). CXCR2 is expressed on fibroblasts, melanomas, and neutrophils. Of these cell types, the neutrophil is known to be recruited to the site of HSV-1 infection in the cornea. These results may be interpreted to predict neutrophil recruitment at day 2 p.i. which coincides with IP-10 mRNA upregulation and that CXCR3 expressing cells infiltrate to peak numbers by day 6 p.i.

These findings are in accordance with those of Araki-Sasaki et al. and Cook et al. who reported IP-10 and CXCR3 message upregulation in the corneas of mice infected with HSV-1 [36, 37]. Taken together, these results confirm that neutrophils are recruited within an appropriate time frame to be a cellular source for IP-10.

Although previous research has begun to explore the role of the neutrophil in production of T-cell-recruiting chemokines at the site of inflammation due to viral infection, other cell types in the corneal model may contribute to IP-10 production. Studies on human corneal epithelial (HCE) and fibroblast (HCF) cells by our research group have described the ability of these cells to express IP-10 after stimulation with proinflammatory mediators such as IL-1α and IFN-γ [38, 39]. Corneal production of IP-10 during the inflammatory response to other viral infections such as respiratory syncytial virus has been established [40]. In the study presented here, we confirm that murine corneas behave in a similar manner and secrete IP-10 constitutively at low levels and significantly elevated levels in response to proinflammatory mediators such as IL-1α produced by physical trauma (e.g., excision of cornea for *ex vivo* incubation). Furthermore, we show that the murine corneal fibroblast cells in isolation produce high levels of IP-10 when stimulated with IFN-γ and to a lesser extent IL-1α. The cells resident in the cornea can thus be demonstrated to have the potential to contribute to IP-10 levels during infection of the cornea.

We have previously demonstrated the ability of murine neutrophils to secrete IP-10 *in vitro* in response to stimulation with IFN-γ but not IL-1α [15]. Here we expand the study to investigate the ability of purified neutrophils to secret IP-10 after *in vitro* stimulation with HSV-1. Neutrophils produce increasing levels of IP-10 in response to HSV-1 stimulation in a dose-dependent manner. The level of IP-10 observed in the assay after stimulation with HSV-1 at a M.O.I of 1 were equivalent to those observed for stimulation with 10 ng/mL IFN-γ, a known signal for IP-10 production in other models [19].

These *in vitro* stimulation experiments provide evidence that within the HSV-1-infected murine cornea there is potential for either resident corneal cells or infiltrating neutrophils to be a source of IP-10. To investigate the relative contribution to IP-10 production by resident corneal cells versus infiltrating neutrophils we conducted *in vivo* cellular depletion experiments. Antibody-mediated cellular depletion of neutrophils, natural killer cells (NK) or $CD4^+$ T cells has been shown to be an effective method for the study of cellular function within inflammatory models [13, 21–23]. We have demonstrated previously that such depletion leads to a reduction in numbers of the cells recruited to the site of inflammation within a DTH model [19]. Here we describe how IP-10 production was significantly reduced in response to reduction in numbers of either neutrophil or NK cells from the host. NK cells are not reported to be a source of IP-10 but do express the receptor for the chemokine (CXCR3) [41]. This suggests that in our study the reduction in IP-10 production observed after NK cell depletion is due to an indirect effect. NK cells are a source of IFN-γ which we demonstrate here to be an effective stimulant for the production of IP-10 by neutrophils. Thus the reduction in IP-10 production after NK cell depletion may be explained in terms of a reduction in IFN-γ stimulation of neutrophils. This conclusion is supported by observations in an IFN-γ gene knockout mouse which demonstrate reduced control of virus replication during corneal infections with HSV-1 [42]. Although $CD4^+$ T cells may also secrete IFN-γ, results from depletion of this cell type do not indicate that the $CD4^+$ T cell is required for IP-10 production in the *in vivo* model within the time frame studied. The depletion studies thus support the hypothesis that neutrophils act as a major cellular source for IP-10 during HSV-1 infection of the cornea and that loss of IP-10 production due to neutrophil depletion cannot be compensated for by IP-10 production by resident corneal cells.

In the final set of data presented we investigated IP-10 production within an *ex vivo* model for human corneal infection by HSV-1. Previous work from our research group has focused on the chemokine and cytokine expression patterns from cultured HCE and HCF cells. In this study, we chose to maintain the potential for communication between these cell types by using corneal buttons comprised of all cell layers normally present in the human cornea. Therefore, we anticipated that the human model would reflect the findings from the murine *in vivo* model. Furthermore, the novel *ex vivo* human model described was designed to permit the study of interactions between resident corneal cells and purified cell populations, such as the neutrophil, in isolation from other cell types normally recruited to the site of inflammation *in vivo*.

In the human *ex vivo* model levels of IP-10 produced by corneas increase significantly when the cornea was incubated in the presence of neutrophils. An equivalent result was observed when the corneas were infected with HSV-1 and incubated with neutrophils. This data suggests that proinflammatory mediators secreted by resident corneal cells in response to either physical trauma (excision) or viral infection lead to neutrophil production of IP-10. Corneal cells are reported to produce a variety of cytokines during response to viral infection including IL-1α, IL-6, CXCL8, and low levels of IFN-γ [43]. Although we found that murine neutrophils secreted high levels of IP-10 due to exposure to HSV-1 (Figure 6) this was not observed to occur to the same extent when human neutrophils were incubated with HSV-1 (Figure 8). Variations between experimental conditions and cellular origin may account for this difference. However, the results from the human *ex vivo* assay do indicate that IP-10 production by neutrophils *in vivo* is stimulated in part by communication from the cornea rather than as a direct response to neutrophil contact with the virus. It should be noted that in the *ex vivo* human model availability of virus for direct contact with the neutrophil is limited when the virus infects the corneal cells (viral eclipse). In conclusion, we present here evidence that neutrophils are a key source of the T-cell-recruiting chemokine, IP-10, during HSV-1 infection of the cornea. Our results suggest that cells resident in the cornea produce proinflammatory mediators in response to HSV-1 infection which recruit and stimulate neutrophils to produce IP-10. The neutrophils thereby contribute to the production

of IP-10 during the early time point after infection (day 2). We hypothesize that this IP-10 leads in part to recruitment of NK cells which promote the inflammatory cascade by further stimulation of neutrophils with IFN-γ to increase IP-10 levels. Peak levels of IP-10 may subsequently recruit T cells leading to viral clearance.

Acknowledgment

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Clinical Study

Support of the Laboratory in the Diagnosis of Fungal Ocular Infections

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This is a retrospective, and descriptive study about the support that the laboratory of microbiology aids can provide in the diagnosis of ocular infections in patients whom were attended a tertiary-care hospital in México City in a 10-year-time period. We describe the microbiological diagnosis in palpebral mycose; in keratitis caused by *Fusarium*, *Aspergillus*, *Candida*, and melanized fungi; endophthalmitis; one *Histoplasma* scleritis and one mucormycosis. Nowadays, ocular fungal infections are more often diagnosed, because there is more clinical suspicion and there are easy laboratory confirmations. Correct diagnosis is important because an early medical treatment gives a better prognosis for visual acuity. In some cases, fungal infections are misdiagnosed and the antifungal treatment is delayed.

1. Introduction

Fungus is a wide group of living organisms very useful in the nature for cellulose degradation and for humans in the antibiotic synthesis and food maturation, and they have coexisted with mankind in its external and internal environment.

Even when fungal ocular infectious diseases are not frequent, actually they are more often described because there are more risky factors like corticosteroid prolonged time treatment or long-lasting broad spectrum antibiotic postsurgical treatment and intravenous drug abuse.

Dimmer in 1913 cited by Francoise [1] published the first case of fungal panophthalmitis. In 1958, Haggerty and Zimmerman [2] described three cases of keratitis in histological studies in patients from Africa with invasion of the fungal infection to corneal epithelium, stroma, endothelium, and reaching vitreous. In Mexico, in 1950, Machado [3] reported the first ocular mycoses, and, in 1969, Gomez-Leal [4, 5] published three systemic mycoses, coccidioidomycosis, mucormycosis, and sporotrichosis with ocular and periocular involvement, with histological studies made by Sadi de Buen and Gonzalez-Ochoa [6, 7].

2. Material and Methods

This is a retrospective and descriptive study of patients attended in Asociacion Para Evitar la Ceguera en Mexico, Hospital "Dr. Luis Sanchez Bulnes" for their diagnosis, medical and surgical attention for fungal ocular infections, in a period of 10 years, from August 2001 to August 2011, based on clinical histories and laboratory records, including all fungal infections attended. Before starting this study, we obtained the permission from the authorities of the hospital to search the clinical history and laboratory records of each patient.

The clinical description was made by slit lamp examination of each patient, and a sample from clinical suspected fungal infection was collected for microscopic examination and culture, with fungal identification according Jones [8].

In order to take the corneal sample, 2 drops of topical anesthetic (tetracaine 5 mgs/mL) were applied before obtaining

smears from the corneal ulcer with heat sterilized Kimura spatula or with the sterile cotton swab (for nasal samples) and were seeded in Petri dishes with culture medium in C streaks and in slant media, for fungus and bacteria cultures. In the same way, three samples were taken for making three smears in the center of each previously cleaned slide marked with a circle made with glass pencil for microscopic observation.

For other samples like conjunctiva, orbital or sclera secretions, the samples were obtained during consultation or surgical procedures by attendant ophthalmologist and sent to the laboratory of microbiology for smears and cultures for anaerobic, aerobic bacteria, and fungus as was referred.

For aqueous and vitreous humors, the samples were taken in the surgical room and sent to the laboratory in the same syringe, in which the samples were collected.

Microscopic examination to identify fungi was made in each case by periodic-acid Schiff (PAS), Giemsa and Gram stains smears according to Prophet [9], and in few cases with calcofluor white and epifluorescent light microscopy. Cultures were made in a wide variety of medium; blood agar, chocolate agar in 4-5% CO_2 ambient at 37° centigrade for bacterial growth, and for fungus Biggy agar slant for *Candida*, Sabouraud dextrose 2%, Sabouraud Emmons both with 0.01% chloramphenicol and without cycloheximide agar slant, with incubation at 27° centigrade and daily observation for a minimum of 3 weeks.

The fungal identification was made by AUXACOLOR 2 (Biorad France) absorption sugars kit for *Candida*, and cell germination forming pseudomycelium. For filamentous and melanized fungus, it was observed the morphology and pigmentation of the colony or in the medium and microcultures for its identification according to Larone [10, 11].

3. Results and Discussion

3.1. Fungal Blepharitis Infections. Fungal infections in the eyelids are less frequent than other fungal ocular infections, they are caused by fungus that live and have harmful effect on the skin named dermatophytes. For its hairy condition, the eyelids and eyebrows are some time involved in fungal infections in children [12] and adults. The dermatophytes described in eyelid infections are the same kind that affects the head skin (Tinea capitis), or in other skin locations (Tinea corporis), or even the intertriginous skin in hands like *Microsporum* or Trichophyton.

We describe a fungal blepharitis case in which at the beginning of infection it is observed like eyelid erythema, and after one week, in the same place, there were little blisters that look opened according with the patient reference, the patient had delayed fungal diagnosis and was treated with topical antibiotic ointment and steroid because it seemed to the first attendant ophthalmologist like one allergic skin reaction. After the desquamation for the breaking of the blisters, the edema remained, and then a *tinea* skin ulcer appeared, it had concentrically involvement in the skin of upper and inferior eyelid with edema, erythematic skin (Figure 1), and loss of eye lashes.

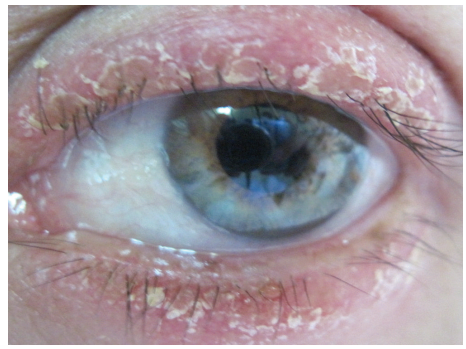

Figure 1: Blepharitis caused by *Trichophyton ajelloi.*

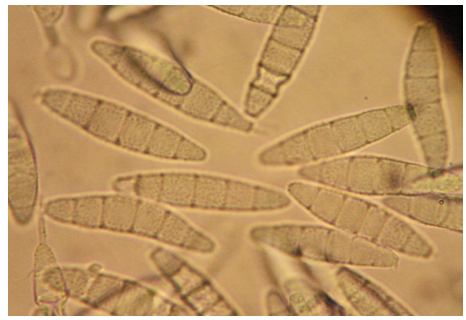

Figure 2: Septate conidia of *Trichophyton ajelloi* from fungal blepharitis from Figure 1 ×400.

The laboratory diagnosis was made by taking a sample of desquamating skin and staining it with periodic-acid Schiff (PAS) technique, the culture revealed after 10 days a white cottony colony that was identified as *Trichophyton ajelloi* (Figure 2).

The medical treatment was made with oral Itraconazole 100 mgs each 12 hs and topic clotrimazole ointment; the normalization of the skin and eyelid were observed after 3 weeks.

3.2. Conjunctivitis by Fungal Infections

3.2.1. Candida Conjunctivitis. Conjunctivitis caused by *Candida* has been described mainly in two life times, in the newborns and school children [13] and in the adults age [14] with the primary infection localized in oral mucosa or vagina.

A follicular-papillary chronic conjunctivitis with no response to topic antibiotic and a slow evolution makes it suspicious to *Candida* conjunctivitis; the patients often have slow response to medical antifungal treatment; in some patients, conjunctiva membranes or pseudomembranes may be observed.

Laboratory diagnosis was made by cultures mainly because the yeast-like cells of *Candida* often are scarce and are overlooked in the smears; the final identification was made by sugar absorption and pseudomycelium formation in a pool of human serum. *Candida albicans, C. parapsilosis,* and *C. tropicalis* had been identified as cause of conjunctivitis.

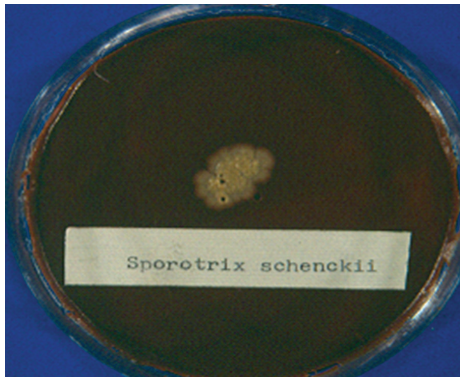

Figure 3: Yeast-like cells colony of *Sporothrix schenckii* isolated from conjunctiva granular tissue in, after five days of incubation in 5% CO_2 ambient in blood agar medium.

3.2.2. Sporotrichum schenckii Conjunctivitis. Conjunctiva infections caused by filamentous fungus are very rare and not frequently diagnosed and published. One caused by dimorphic fungus *Sporotrichum schenckii* was reported in Japan with the diagnosis made for histological study, over bulbar conjunctiva and with a good response to topical fluconazole and oral potassium-iodine [15]. In Mexico, we attended one case culture proved, in 19-year-old female patient, the infection was located in inferior tarsal conjunctiva in right eye and external angle, with a 4 mm by 3 mm zone of granular tissue over the conjunctiva and edema around; the granular conjunctivitis started two months before being attended, preauricular enlargement of lymph node, and no systemic involvement was observed in her first consultation. Fungal culture revealed in blood agar and 5% CO_2 white-yellow colonies formed by yeast-like cells (Figure 3), and after 10 days in Sabouraud dextrose agar grew a cottony white-gray and finally black colony that was identified by its colony morphology and microculture characteristic as *Sporothrix schenckii* [16].

The patient was treated with oral potassium-iodine solution, and the response was very good for conjunctiva symptoms at third day, and total cure was obtained eleven days after beginning the treatment.

3.3. Fungal Keratitis. Some keratitis are caused by fungus that live freely in the teleomorphic form in the environment like *Fusarium* or *Aspergillus*; this fungus in laboratory cultures shows asexual spores with meiotic or anamorphic cellular division. Cornea can be invaded for three kind of fungus; white filamentous, filamentous-melanized, and yeast-like *Candida*. Each kind of this fungus owns to groups and families in constant reclassification. Nowadays, there is an increase of new cases and reports related to soil contaminated corneal trauma, contaminated contact lens [17], inadequate disinfectant contact lens solutions, topical steroid abuse, and dry eye [18].

The traumatic antecedent risks are more often referred in patients in developing countries. Meanwhile, the use of contact contaminated lens was as main risk referred in developed countries, and the contaminant fungus reported was *Fusarium solani*, *Acremonium (Cephalosporium)*, *Paecilomyces*, *Candida*, *C. tropicalis*, *Curvularia, Alternaria*, and *Aspergillus* according to Wilhelmus [17].

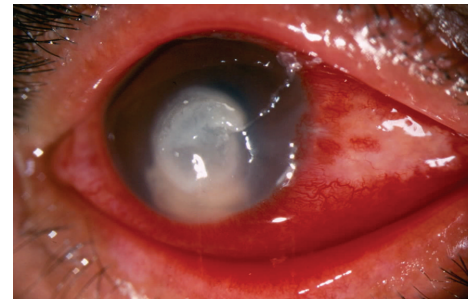

Figure 4: Fungal keratitis caused by *Aspergillus fumigatus*.

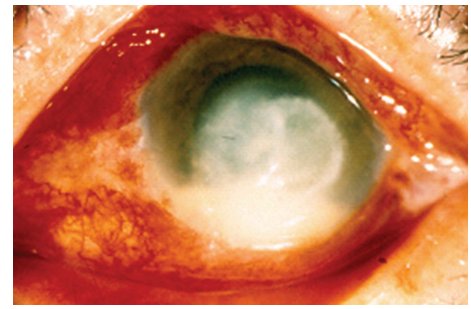

Figure 5: Fungal keratitis caused by melanized filamentous *Alternaria spp.*

Clinical signs and symptoms are no different from the ulcers caused by white filamentous and filamentous melanized fungus. The slow evolution, risk factors and the clinical signs are important facts for the diagnosis, the smears and cultures aid the final approach to diagnosis (Figures 4 and 5). In one serial of 219 keratitis cases studied in our center for eye care, 75.3% were male and 24.6 % were female patients with ages ranging from 8 to 94 year old and 46 median years old, 36% cases were referred previous corneal trauma, and surgical trauma in 5.4% developed postsurgery mainly caused by *Candida*.

3.3.1. Fusarium. In the corneal samples, smears with PAS stain, *Fusarium* showed septate fungal cells (hyphae) (Figure 6) indistinguishable to other septate fungus. The cultures grew in 48 to 72 hours in blood agar at 37° centigrade developing cottony white colonies.

On Sabouraud dextrose 2% and Sabouraud Emmons mediums without cycloheximide, a white or red-yellowish color are developed at the reverse of the colony at 4 or 5 days of incubation. In the microculture technique identification, they showed banana-like macroconidia with 3-4 cells division and round to oval microconidia and were recognized according to Nelson [19].

In our patients' keratitis cases serial study, *Fusarium solani, F. dimerum, and F. oxysporum* were identified in 37.7%.

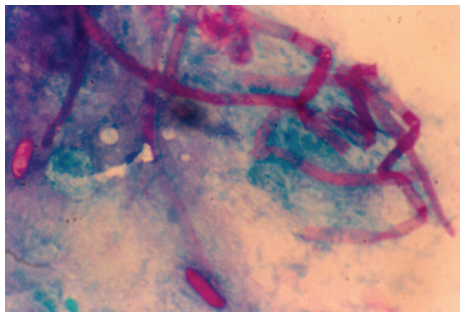

Figure 6: Pas stain of septate hyphae in corneal sample smears ×400.

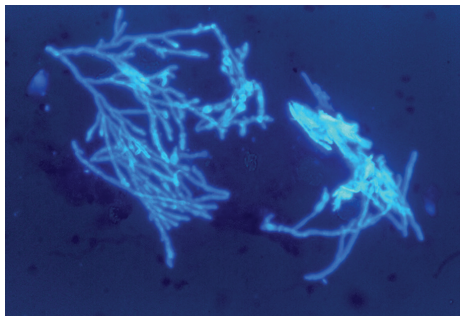

Figure 7: Microscopic view of fungal cells in a corneal sample smear with calcofluor and epifluorescent light ×400.

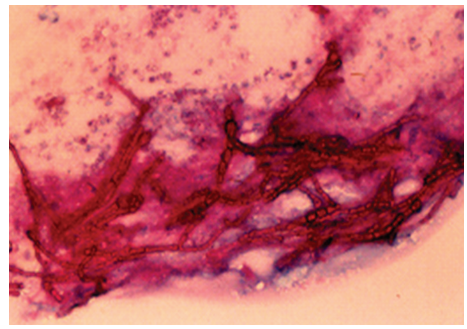

Figure 8: PAS stain in smear from keratitis caused by melanized fungus ×400.

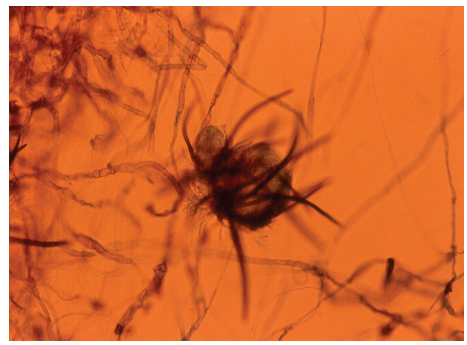

Figure 9: *Chaetomium globosum* isolated from a fungal keratitis in an agronomist patient ×200.

3.3.2. Aspergillus. In the corneal sample smears, the hyphae showed septate cells and, in calcofluor stain technique, were indistinguishable to other moulds (Figure 7). In cultures, *Aspergillus* showed cottony white-green, or green-grey or brown-black color upon the specie colonies, with its characteristics conidiophores monoseriate or biseriate for its identification, and round to oval conidia. In our patient serial study, *Aspergillus* was isolated in 26% of keratitis cases. *Aspergillus fumigatus*, *A. nidulans*, *A. flavus*, *A. niger*, and *A. glaucus* were the most identified species.

3.3.3. Filamentous Melanized Moulds. Keratitis caused by filamentous melanized fungi in some cases showed brown-color over the corneal ulcer because the fungal cells are brown-color too (Figure 8).

Filamentous melanized fungi were isolated from 39% of the keratitis studied [20] and were identified *Curvularia geniculata*, *Cladosporium carrionii*, *Alternaria spp*, *Phialophora spp*, *Exophiala spp*, *Wangiella spp*., *Scytalidium lignicola*, *S. dimidiatum, Phialemonium*, and, in one case, *Chaetomium globosum* (Figure 9).

In our serial keratitis studied, the most frequent fungus involved as cause of infection was Fusarium in 37.7%, and *Aspergillus* in 26%. Traumatic antecedents were referred in 36%. In Madurai, India, Srinivasan reported in 139 fungal keratitis 47% caused by *Fusarium*, and 17% caused by *Aspergillus*, and trauma referred in 46.8%, our serial patient seemed in the relation of *Fusarium* and *Aspergillus* to the Indian author [21].

3.3.4. Candida spp. Candida was isolated in 16% of those patients diagnosed with fungal keratitis. *Candida albicans, C. parapsilosis, C. glabrata*, and *C. tropicalis* were identified (Figures 10 and 11).

Candida albicans keratitis was identified in an 8-month-old female.

In temperate zones like Philadelphia Tanure, reported 24 keratitis cases in adults, and *Candida albicans* represented 45% of the fungus isolated in his serial study, and *Fusarium* only 25% [22].

3.4. Mycotic Endophthalmitis. Fungi that cause endophthalmitis reach vitreous by two ways, by trauma or surgical trauma or by endogenous way in the middle of a transient fungemia derivate from other site fungal infection, and some fungal cells reach the artery or venous retinal system.

In our serial study, we found a low frequency of fungal endophthalmitis, in 234 samples of aqueous humor and 422 samples of vitreous humor from 369 patients studied for endophthalmitis diagnosis in ten years; we obtained 189 positive cultures for bacteria or fungus, the number of fungal culture positive endophthalmitis were 17, all obtained in vitreous samples, and the percent of fungal endophthalmitis diagnosed by cultures, related to positive bacteria or fungus samples, was 8.99% (17/189).

The antecedent risks and endophthalmitis diagnostic 2/17 (11.7%) were caused for ocular trauma and foreign body in retina, confirmed with Paecilomyces in both cases;

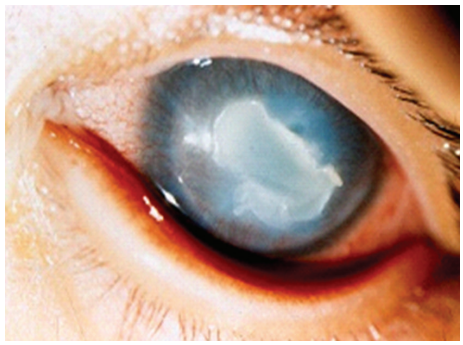

Figure 10: *Candida albicans* keratitis in an 8-month-old female.

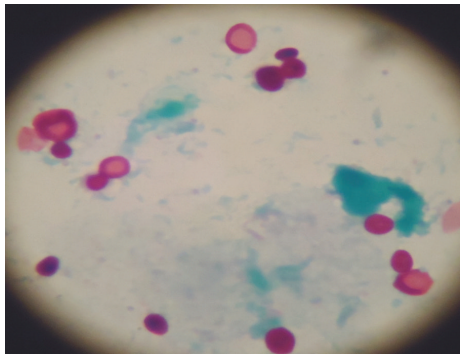

Figure 11: Yeast-like cells of *Candida albicans* in a smear from fungal keratitis sample stained with PAS ×1000.

previous surgical trauma in five cases 5/17 (29.4%) *Candida*, *Aspergillus*, *Acremonium*, and *Phoma*; in one case 1/17 (5.8%), the endophthalmitis developed after *Aspergillus niger* fungal keratitis due to cornea trauma. Endogenous endophthalmitis was 9/17 (52.9%) caused by *Candida albicans* in five *cases*; one case by *Candida dubliniensis* and one case by *Candida tropicalis*, in one case was isolated *Penicillium citrinum*, and one case with fungus cells in the smear was isolated *Scopulariopsis spp*, in the vitreous sample. In any endogenous endophthalmitis case described, the hemoculture was positive.

From this series of patients, we describe one case of post-PRK (Photorefractive keratectomy) fungal keratitis. After a wide central corneal fungal keratitis, it was diagnosed (Figure 14), the patient was submitted to topical treatment with natamycin 5% suspension topical drops (Miconacina Sophia México); the therapeutic response to medical treatment was acceptable, and, for visual reasons, the patient was submitted to penetrate keratoplasty (PKP). Ten days after PKP, the patient developed a fungal endophthalmitis diagnosed by smears and cultures of aqueous and vitreous samples (Figures 12, 13, and 14).

The fungus isolated was *Phoma* (Figure 15), a rare, fungus classified in genus Peyronellaea [23] associated with an endophthalmitis case described by Errera [24]. Our patient was submitted to intravitreous and oral voriconazole treatment, and the final visual acuity was diminished by retinal detachment.

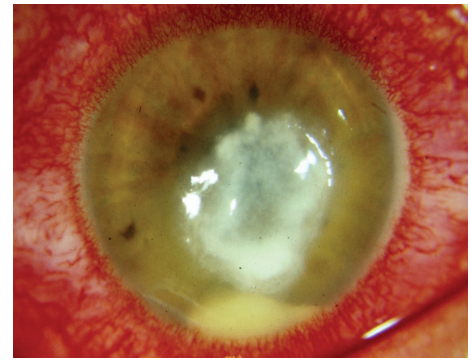

Figure 12: Fungal keratitis post-PRK hypopyon and central ulcer with satellite lesions.

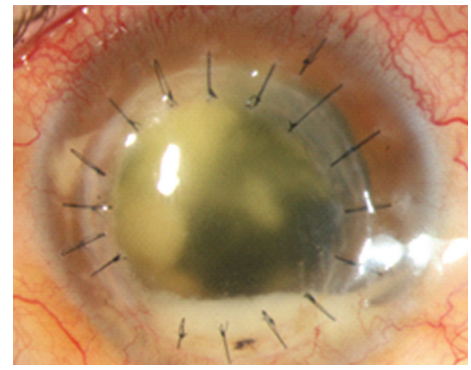

Figure 13: In the same case, in Figure 14, after corneal transplant for visual acuity reason, the patient developed endophthalmitis signs.

3.5. Fungal Sclera Infection. Sclera fungal infections are very rare events, and, in our series of cases, we described an histoplasma sclerotic infection in an immunosuppressed 47-year-old female, who was being treated for rheumatoid arthritis and dermatomyositis diagnosed 3 years ago, and treated with oral methotrexate 10 mgs weekly, azathioprine 50 mgs, and oral prednisolone 10 mgs both daily, was attended in our hospital by a red and swelled eye, with diminished ocular vision, foreign body sensation in left eye, and one sclerotic lesion in the same eye.

At the slit lamp ocular examination, it was observed: visual acuity: left eye CF at 4 meters and corrected 1.5/10, important ecchymosis and induration in the eyelid.

In conjunctiva, hyperaemia, chemosis, and ecchymosis evaluated each in ++++ and a sclerotic lesion of 4 mm located in the external angle, covered by gray-white secretion (Figure 16), were diagnosed as infectious scleritis. One sample was taken for bacteria and fungus cultures and smear as previously described.

The smear with Giemsa stain showed intracellular yeast like forms in hystiocytes, and in cultures after 12 days of incubation, a cottony white colony grew in Sabouraud-Emmons media (Figure 17) and in microculture shown *Histoplasma* conidia (Figure 18), in a second sample taken from the same sclerotic ulcer, the cultures had the same microbiologic results.

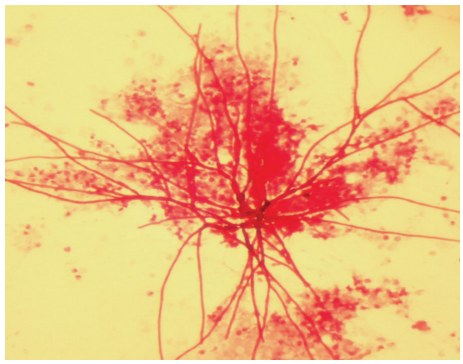

Figure 14: Hyphae and inflammatory cells observed in aqueous humor from case in Figure 14 ×1000.

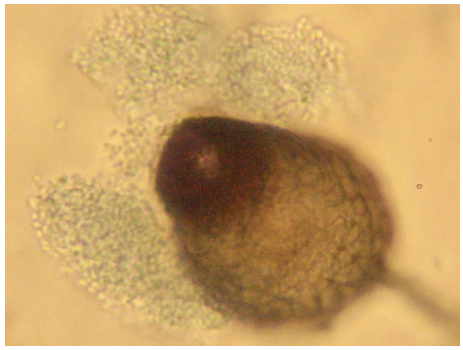

Figure 15: Pycnidial forms of genus Peyronellaea that identified *Phoma,* obtained in the aqueous and vitreous samples of the patient from Figure 14 ×400.

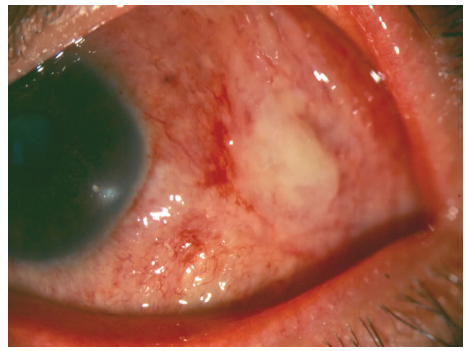

Figure 16: Scleritis due to *Histoplasma capsulatum* in a female immunosuppressed patient.

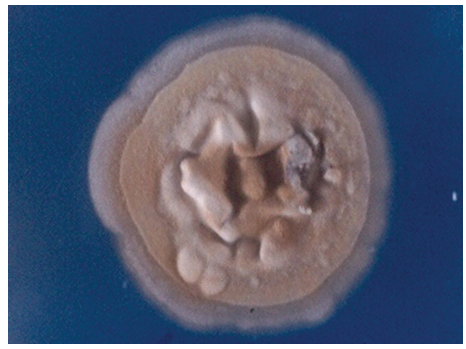

Figure 17: *Histoplasma capsulatum* tan colored colony after 20 days of incubation in Sabouraud-Emmons agar obtained from sclera sample culture.

Because of the laboratory smear report, oral Itraconazole 100 mgs/day was added for treatment; prednisolone was diminished to 25 mgs by mouth each day for leucopenia detected. The ocular response to itraconazole treatment was very good, within 8 days after starting oral itraconazole. Laboratory informed the identification of fungal colonies; *Histoplasma capsulatum* var. *capsulatum* and, for the PCR identification, HC 100 protein (110 Kd) and M protein were used.

Ocular histoplasmosis culture proved is not often described; there are some endophthalmitis cases referred in immunosuppressed patients [25]. Sclerotic infections *Histoplasma capsulatum* cultures proved had not been reported before.

In Mexico, there are serologic epidemiologic studies about the infections in some risky areas [26–28]. The female patient described in histoplasmosis scleritis case lives in an endemic template zone in Morelos State, where the fructivorous bats population are high and the fruit trees are near around the living homes. We think that this together with her immunosuppression was the risk facts for the ocular infection developed.

3.6. Fungal Orbital Infections. In immunosupressed patients, orbital subcutaneous mucosal infections are often caused by *Aspergillus*, *Mucor*, *Absidia*, and *Rhizopus* that are moulds that belong to the class Zygomycetes. The terms mucormycosis or zygomycoses are used to refer to the subcutaneous or deep infections like rhinoorbital, rhinocerebral, cerebral, gastrointestinal, and pulmonary mycose caused any one of these moulds. The term aspergillosis is used for pulmonary, paranasal, sinusal, or rhinoorbital fungal infrequent infection caused by diverse species of *Aspergillus* [29].

We described the microbiologic diagnosis of one immunosuppressed patient for a kidney transplant that presented a mucormycosis that began like nasal sinusitis with orbital inflammatory involvement caused by *Rhizopus arrhizus* (formerly *Rhizopus oryzae)* (Figure 19); 30 days after the sinusitis debridement surgery and antifungal treatment, the same patient, because of work risk, was overinfected and developed a second nasal sinuses severe inflammation and a second fungal infection caused by *Aspergillus niger*.

In the first sinus debridement tissue sample, no septate hyphae was observed in PAS stain, and the culture in Sabouraud Emmons 2% without cycloheximide agar slant medium yields a fast growing fungus white-gray colony identified like *Rhizopus arrhizus* (Figure 20); in the second surgery for debridement, the samples showed a mixed fungus culture in Sabouraud Emmons without cycloheximide, and, in blood agar Petri dishes, streaked for isolation, one colony was white-gray (*Rhizopus arrhizus*) and the other was white and

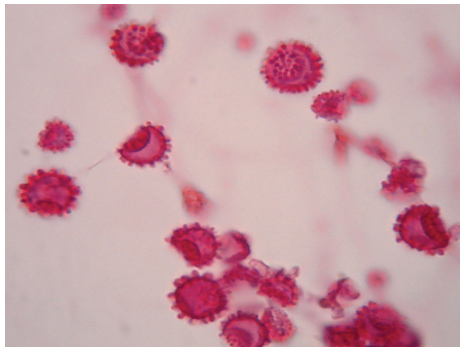

Figure 18: Microscopic view of microculture of *Histoplasma capsulatum* PAS stained. From scleritis case sample, described in Figure 16 ×400 magnification.

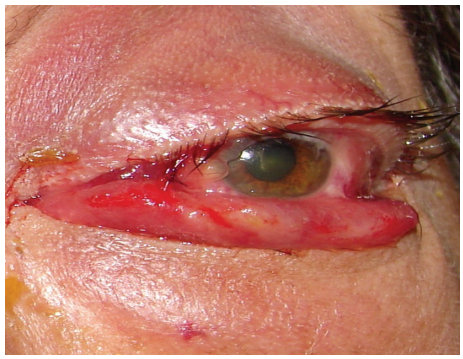

Figure 19: Patient showed an evident quemosis, and orbital inflammatory involvement of fungal sinusitis, diagnosed as mucormycosis.

3 days after turned into black on the surface, and, finally, it was identified by microculture technique like *Aspergillus niger* [30].

4. Conclusion

Ocular fungal infections fortunately are infrequent; its diagnosis, today, is easy because there are laboratory facilities for its confirmation. Some fungal keratitis are distinguishable clinically from other infectious keratitis, but clinically fungal endophthalmitis are difficult to differentiate from bacterial endophthalmitis. Fungal ocular infections treatments are difficult to manage because some antifungal drugs are not water soluble and do not penetrate in optimal concentrations into the tissues where they are useful, some like Amphotericin B and its derivatives are toxic for the delicate ocular tissues.

For these reasons, an early clinical and laboratory diagnosis are very important for a better final visual prognosis. Preventive measures avoiding ocular trauma and adequate contact lens disinfection can diminish fungal infections in cornea. Prophylactic antifungal treatment in intensive care for patients whom are with long-lasting assisted respiratory or catheters, using an oral or intravenous dose of triazoles for fungal endophthalmitis concomitant to long-term and broad spectrum antibacterial treatment, is a good project to reduce the incidence of these intraocular infections.

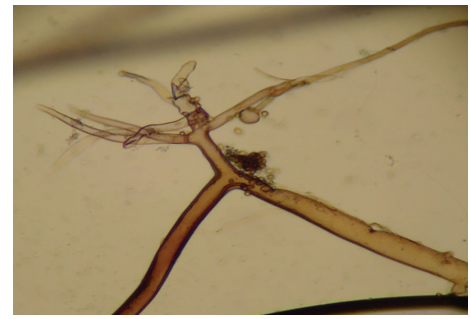

Figure 20: *Rhizopus arrhizus* in microculture, isolated from first sample of tissue nasal sinus debridement in patient from Figure 19 ×400 magnification.

Conflict of Interests

The authors have no commercial or other conflict of interests in any of the products and techniques mentioned in this study.

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The authors are in debt with all ophthalmologists of the Asociación para Evitar la Ceguera en México Hospital who attended the patients whose cases were mentioned in this study, to the Pathology Laboratory for its invaluable help, to the Head of the UNAM Histoplasma Mycology Laboratory M Lucia Taylor, and to Mrs. Elia Portugal for her kind and cooperative editorial work.

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Review Article

Cataract Surgery in Uveitis

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Cataract surgery in uveitic eyes is often challenging and can result in intraoperative and postoperative complications. Most uveitic patients enjoy good vision despite potentially sight-threatening complications, including cataract development. In those patients who develop cataracts, successful surgery stems from educated patient selection, careful surgical technique, and aggressive preoperative and postoperative control of inflammation. With improved understanding of the disease processes, pre- and perioperative control of inflammation, modern surgical techniques, availability of biocompatible intraocular lens material and design, surgical experience in performing complicated cataract surgeries, and efficient management of postoperative complications have led to much better outcome. Preoperative factors include proper patient selection and counseling and preoperative control of inflammation. Meticulous and careful cataract surgery in uveitic cataract is essential in optimizing the postoperative outcome. Management of postoperative complications, especially inflammation and glaucoma, earlier rather than later, has also contributed to improved outcomes. This manuscript is review of the existing literature and highlights the management pearls in tackling complicated cataract based on medline search of literature and experience of the authors.

1. Introduction

One of the most daunting tasks for an ophthalmic surgeon is the management of complicated cataracts. Cataract in uveitis may develop as a result of the intraocular inflammation per se, from chronic corticosteroid usage or more often from both [1]. The incidence of cataract in uveitis varies from 57% in pars planitis [2] to 78% in Fuchs heterochromic iridocyclitis (FHI) [3].

Cataract surgery in uveitic eyes is challenging and can present with many unforeseen intraoperative complications. Two decades ago, the outcome of surgery in these eyes was guarded and often confounded by postoperative complications such as severe inflammation, hypotony, and even phthisis bulbi. However, with modern day cataract surgical techniques, this is seldom the case. With improved understanding of disease processes, optimization of immunosuppression for perioperative control of inflammation, minimally invasive surgical techniques, availability of biocompatible intraocular lens material and design and surgeons trained in performing complicated cataract surgeries and anticipatory management of postoperative complications [4], the outcome has been maximized. Ocular morbidity in patients undergoing complicated cataract surgery is now limited to those cases that have pre-existing changes in the retina or optic nerve such as irreversible macular scarring or optic nerve atrophy. The fundamentals of cataract surgery in patients with different forms of uveitis have been recently reviewed by numerous authors [1, 4–17]. This paper presents a review of the existing literature on this topic and proposes a set of management pearls in tackling complicated cataract based on the literature review and authors' combined experience.

Extensive literature search using OVID medline search engine and all available library databases was employed with references cross-matching to obtain all peer reviewed articles published on cataract surgery in uveitis. The literature search

included all relevant published studies on cataract surgery in uveitis since 1960 in English language.

2. Historical Perspective

Evolution of cataract surgery in uveitis. Until the advent of corticosteroids in the early 1960s, ocular inflammation was difficult and often impossible to control, and articles discussing the results of cataract extraction in inflamed eyes reported a high incidence of severe complications [4, 6, 7, 10].

In many cases, the complications resulted in marked reduction of vision or even loss of the eye [11]. More recent publications have reported a considerable decrease in the incidence of intraoperative and postoperative complications during cataract extraction in uveitis [4–7, 12]. The most likely reasons for this vast improvement would appear to be the increased ability to control inflammation perioperatively and the rapid evolution in microsurgical techniques that has occurred in recent years.

3. Review of Current Literature

3.1. Cataract Extraction in Different Uveitic Entities. Several factors must be kept in mind when assessing the current literature on cataract extraction in patients with uveitis. Inflammatory squealae may develop at relatively predictable rates in eyes with a given inflammatory syndrome [4–7, 11–17]. However, these rates do vary markedly among syndromes. It is therefore important to consider each syndrome separately when assessing the results and complications encountered following cataract extraction.

3.1.1. Cataract Surgery in Patients with Fuchs Heterochromic Iridocyclitis (FHI). By far the largest volume of information regarding cataract surgery in uveitis patients concerns those with FHI. The uveitis tends to be low grade and chronic, posterior synechiae rarely form, and patients are often unaware of the disorder until complications develop or the inflammation is discovered during a routine eye examination [18]. If patients are symptomatic, the two most common symptoms at the time of presentation are blurred vision as a result of cataract formation and vitreous floaters [18, 19]. The reported incidence of cataract formation in FHI syndrome ranges from 15% to 75%, with the vast majority of series reporting an incidence of around 50% [3, 4, 11, 13, 18, 19]. Most cataracts are of the posterior subcapsular type, with the remainder being cortical or mixed [18].

Numerous studies of cataract extraction in FHI have reported insignificant intraoperative and postoperative complications. To summarize, the recurring complications seen following cataract surgery in FHI, in order of reported frequency, were hyphema, progressive vitreous opacification, glaucoma, and spontaneously resolving vitreous hemorrhage. Other complications such as retinal detachment, extensive synechiae formation, and corneal edema were reported by some authors [3, 18, 19].

The reported incidence of intraoperative and postoperative hemorrhage varied from 3.6% to 76%. Recent articles report a much lower incidence of hyphema than was recorded previously, perhaps a result of the use of improved microsurgical techniques. Javadi and colleagues had safe outcomes with phacoemulsification and in-the-bag intraocular lens implantation in FHI, achieving a post operative visual acuity of 20/40 or better in all 41 eyes in their series. Vitreous haze was the major cause of postoperative visual acuity of less than 20/20. In the follow-up period of 17.8 ± 8.7 months, the only complication was PCO, which developed in six (14.6%) eyes [18]. The most visually significant complication of cataract extraction in FHI eyes appears to be the development of glaucoma. Permanent elevation of IOP was reported to develop at some time following the operation in 3% to 35% of these eyes [13]. The review of the literature reveals that, initially, control of intraocular pressure can be achieved by medication alone, but eventually up to 70% of these patients will require filtering surgery [13].

3.1.2. Cataract Surgery in Patients with Juvenile Idiopathic Arthritis (JIA). Not unlike inflammation associated with FHI, the chronic nongranulomatous anterior segment inflammation associated with this condition is often asymptomatic until complications supervene. However, the complications arising from this syndrome are more severe. The literature indicates that commonly encountered complications include band keratopathy, extensive posterior synechiae, and hypotony or glaucoma, in addition to cataract.

Rehabilitation of 40–60% eyes with JIA that develop cataract is considerably more difficult than that in eyes with FHI [20–31]. Numerous studies have reported the poor post operative outcome of conventional cataract extraction even without intraocular lens implantation. The largest series of patients with JIA who had cataract extraction was reported by Kanski and Shun-Shin [32]. Out of 162 eyes, 61 had the cataract removed by needling and aspiration, and 101 had lensectomy and limited anterior vitrectomy. Vision was hand motion or less in l5% of the lensectomized eyes, 20/400 to count fingers in 30%, and better than 20/60 in 56%.

It was not until the important concept of adequate immunosuppression aimed at zero tolerance of inflammation and abolishment of every cell was strongly advocated and supported by clinical studies [22, 33] that patients with JIA undergoing cataract surgery saw better visual outcomes. In his landmark paper, Foster described the intensive use of preoperative and postoperative corticosteroids and reported visual acuities of 20/40 or better in 67% of patients and no major intraoperative or postoperative complications [22]. Subsequently several studies reported successful postoperative outcome with limited complications with perioperative and postoperative immunosuppression. Treatment with systemic, topical, and periocular steroids is recommended during the perioperative period for all eyes with uveitis associated with JIA that undergo cataract

extraction. Surgery should be delayed until the anterior chamber is free of inflammatory cells ("flare" will persist).

The addition of a limited vitrectomy in combination with lens removal resulted in a decrease in the incidence of phthisis from 25% to 2% in a series described by Kanski [34]. Another similar study revealed the beneficial outcome with vitrectomy in patients with JIA-associated uveitic cataract [35].

Successful use of intraocular lens implantation in uveitis was reported by Probst and Holland in 1996 wherein visual acuity of 20/40 or better was achieved in eight eyes at an average follow-up period of 17.5 months; however the study was limited by very small number of patients [26]. The patients were operated at around a mean age of twelve years, which was almost five years after the diagnosis of JIA in this study as against the midteens in other studies.

A more recent study by Kotaniemi and Penttilä in 2006 reported good postoperative outcome following intraocular lens implantation in patients with juvenile idiopathic arthritis-associated uveitis where cataract surgery with intraocular lens implantation was performed in 36 eyes and the mean postoperative follow-up period was 3.3 years. The visual result was good (>0.5) in 64%, moderate (0.3–0.5) in 11%, and poor (0.3) in 25% eyes. Secondary cataract developed in 16 eyes but in none of the eyes with primary posterior capsulotomy and anterior vitrectomy. Secondary glaucoma developed in 18 eyes, retinal detachment in 2 eyes, cystoid macular edema in 16 eyes, and band keratopathy in 12 eyes [36].

Another major study published in 2009 by Quiñones et al. looking at cataract extraction in children with chronic uveitis with 21 out of 34 children having JIA reported good tolerance of intraocular lens in JIA patients and good postoperative visual outcome with optimal control of inflammation with immunomodulatory therapy. The average followup reported in this study was more than four years [33].

Recently published study by Ganesh and colleagues analysed ten eyes of 7 patients who had phacoemulsification with IOL implantation done by a single surgeon. A heparin surface modified IOL was used in 7 eyes and a foldable acrylic IOL was used in 3 eyes. At final followup, 70% of eyes had a visual acuity of 20/40 or better and 30% had improved visual acuity to 20/60. Posterior capsular opacification was found in 2 eyes and anterior capsular fibrosis in 1 eye [37].

Most JIA patients are in the amblyogenic age when they develop cataracts and the surgeon must bear this in mind when planning cataract surgery. For this reason, cataract surgery cannot be withheld or delayed for too long whilst battling to keep the eye quiescent in preparation for the operation. Furthermore, primary capsulotomy with limited anterior vitrectomy may be considered in children under the age of six to eight years as performing YAG capsulotomy in the postoperative period for posterior capsular opacification may be difficult in a very young child.

A number of factors combine to make cataract surgery more hazardous in these patients. These eyes have a marked tendency to form synechiae [22]. Implantation of an IOL into the capsular bag at the time of cataract removal often results in reformation of posterior synechiae and development of membranes over the IOL, resulting in cocooning of the IOL [26]. This may results in malignant glaucoma if the iris is plastered back onto the anterior capsule. If seclusion or occlusion pupillae occur, secondary pupil block glaucoma develops warranting a surgical peripheral iridectomy [26]. With cyclitic membrane formation, traction of the ciliary processes and ciliary body result in hypotony and phthisis bulbi if surgery is not done early. Although glaucoma is common in this syndrome due to the chronicity of inflammation, with a reported incidence of about 25% in most studies, some eyes are hypotonous by the time cataract extraction is contemplated [22, 35]. In addition to making surgery technically more difficult, hypotony is associated with an increased risk of postoperative choroidal effusion, macular edema, and phthisis. As a result of the high risk of complications that may develop following surgery, there is still controversy surrounding the implantation of an IOL at the time of surgery [29]. These complications are still very real despite the development of biocompatible IOL materials. Thus, even with successful cataract surgery, the outcome of cataract surgery in JIA patients is often limited by the state of the optic nerve and the macula and may be compromised by the presence of band keratopathy [21, 22, 24].

3.1.3. Cataract Surgery in Patients with Intermediate Uveitis. Inflammation in eyes with pars planitis is limited primarily to the posterior segment, although mild anterior chamber activity is present in some cases. This contrasts markedly with the inflammation seen in Fuchs syndrome or JIA-associated uveitis, which is located primarily anterior to the lens. As the anterior chamber is largely free of inflammation in intermediate uveitis, synechiae seldom develop and the incidence of glaucoma is low [2]. As chronic inflammation persists in close proximity to the lens, cataracts eventually develop in 40% of patients [2]. Lens opacities first develop as a diffuse haze in the posterior subcapsular region. A large percentage of the cataracts remain at this stage; only half of the cataracts in one large series eventually became visually significant [2].

Macular edema is the major complication encountered following surgery in pars planitis patients and is the most important cause of poor vision. It has been seen to some degree in almost half of the eyes that undergo cataract extraction and is responsible for 80% of the eyes with less than 20/40 vision. Generally, few other complications are seen, and inflammation appears to remain under control following surgery. The incidence of glaucoma after cataract surgery in uveitis averages around 10%, which closely parallels the natural rate of glaucoma in this syndrome [7]. Numerous studies have reported varying results of cataract extraction in patients with intermediate uveitis [2, 4, 20, 38–42]. A possible reason for the varied outcomes is that intermediate uveitis can take on a variable clinical course, with approximately a third of all patients having a severe prognosis despite therapy. There have been a few studies which showed good postoperative outcome with vitrectomy

along with cataract extraction in patients with chronic intermediate uveitis [43–47].

A large study by Ganesh and colleagues in 2004 [38] analyzed the outcome of phacoemulsification with intraocular lens implantation in 100 eyes with intermediate uveitis. In this study, 91% of eyes showed a favorable visual outcome at average followup of 19.67 months. The major complications reported by authors in this study were significant posterior capsular opacification which occurred in 10%, CME in 50%, reactivation of intermediate uveitis in 51%, IOL deposits in 29%, IOL decentration in 1%, and anterior capsule fibrosis in 14%. The three most frequent causes of poor visual recovery were CME, submacular fibrosis, and epiretinal membrane. The authors concluded that phacoemulsification with IOL implantation in eyes with pars planitis was safe and led to good visual outcomes in most cases. They attributed the success to control of inflammation, meticulous surgery, in-the-bag IOL implantation, and vigilant postoperative care.

3.1.4. Cataract Surgery in Behcet's Disease. Cataract formation is the most common anterior segment complication after recurrent inflammation, occurring in up to 36% of cases [1]. It was reported that the postoperative visual acuity was found to be significantly lower in eyes with BD than in those with idiopathic uveitis because of the severe posterior segment complications, mainly optic atrophy [48].

Surgery is indicated whenever visual improvement can be expected and the eye has been free of inflammation for a minimum of 3 months. Operating on eyes with cataract and uveitis has been previously reviewed by Foster and associates [8]. Their recommendations for a successful cataract surgery and for minimizing the postoperative uveitis are as follows.

Uveitis should be inactive for at least 3 months preoperatively, systemic and topical steroids should be used prophylactically for 1 week preoperatively and continued postoperatively, immunosuppressive drugs should be continued, complete removal of cortical material should take place, and one-piece PMMA posterior chamber intraocular lens should be used if the patient and the surgeon understand the special nature of this surgery, its risks, and the prognosis for success.

In another paper by Berker et al., the authors reported results of phacoemulsification and intraocular lens implantation in patients with Behcet's disease [49]. They reported 72.5% of eyes had improvement in vision after surgery. However, the vision got worse in 17.5% of the eyes. Most frequent complication reported by them was posterior capsular opacification in 37.5% of eyes. Other complications were posterior seynchiae and severe inflammation. Posterior segment complications such as epiretinal membrane formation, cystoid macular edema, and optic atrophy were also reported by the authors.

3.1.5. Cataract Surgery in Patients with Idiopathic and Other Forms of Uveitis. This group includes patients with uveitis associated with sarcoidosis, toxoplasmosis, Vogt-Koyanagi-Harada (VKH) syndrome, sympathetic ophthalmia, and other types of uveitis. Duke-Elder [50] and Smith and Nozik [51] both reported based on anecdotal evidence that such patients do well following conventional surgery, as long as inflammation has been absent for at least two to three months preoperatively. Moorthy et al. [52] performed cataract surgery in 19 eyes of VKH. 68% of eyes had best corrected visual acuity (BCVA) of >6/12. The most common reason for BCVA <6/12 was pigmentary disturbance in macula. In 1983, Reynard and Meckler [53] reported the results of cataract extraction in six sympathizing eyes of patients with sympathetic ophthalmia. All eyes showed minimal inflammation at the time of surgery, two eyes underwent intracapsular cataract extraction, three extracapsular cataract extraction, and in one case the cataract was needled. Following surgery, all the eyes required steroid treatment during followup to control recurrences of inflammation. Uncontrolled inflammation led to the formation of cyclitic membranes or phthisis in three eyes in spite of corticosteroid therapy. Two eyes achieved visual acuity better than 20/40 during the follow-up period, which ranged from one to 23 years. The three eyes with severe postoperative inflammation retained only light perception vision; one eye, with chronic inflammation and macular edema, retained 20/100 vision.

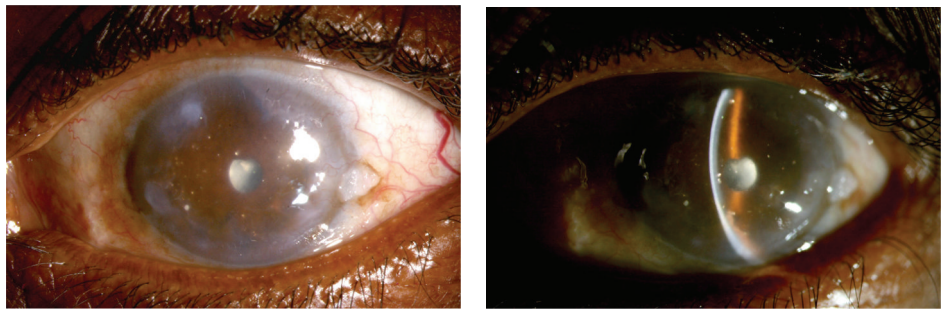
Akova and Foster [54] analyzed results in 21 eyes of sarcoidosis. 61% eyes achieved a stable visual acuity of >6/12. In 2004 Ganesh et al. [55] reported results of cataract surgery in 59 eyes of VKH and found BCVA improvement by one or more lines on Snellen's chart in 40 (67.79%) eyes. PCO was seen in 38 (76%) eyes, followed by optic atrophy and subretinal gliosis.

Fox et al. [23] described 16 patients with various types of uveitis associated with ankylosing spondylitis in 5 and inflammatory bowel disease in two. All patients had less than 0–2 anterior chamber cells for at least three months preceding surgery. Cataracts were removed by extracapsular techniques, including phacoemulsification, and 14/16 eyes had posterior chamber intraocular lenses implanted. Vision improved in all cases, with most eyes achieving 20/40 or better visual acuity. Few complications were noted, the most serious appeared to be the development of posterior synechiae and in 6/14 eyes (43%), macular pathology was seen postoperatively.

4. Current Guidelines for the Management of Uveitic Cataract

4.1. Clinical Evaluation of Complicated Cataract and Associated Uveitis. The eye in which visual loss is mainly attributable to cataract formation is most likely to benefit from cataract surgery. The outcome of surgery depends upon several factors, namely the uveitic diagnosis, proper perioperative management and meticulous surgery. The specific uveitic diagnosis is of paramount importance also when planning the surgical strategy [4], such as determining whether an intraocular lens should be implanted or not.

Diseases that spare the posterior segment generally have a better prognosis than those that affect the macular and/or optic nerve. Acute uveitic syndromes tend to be associated with better outcomes than chronic uveitis. Thus, JIA patients,



(a) (b)

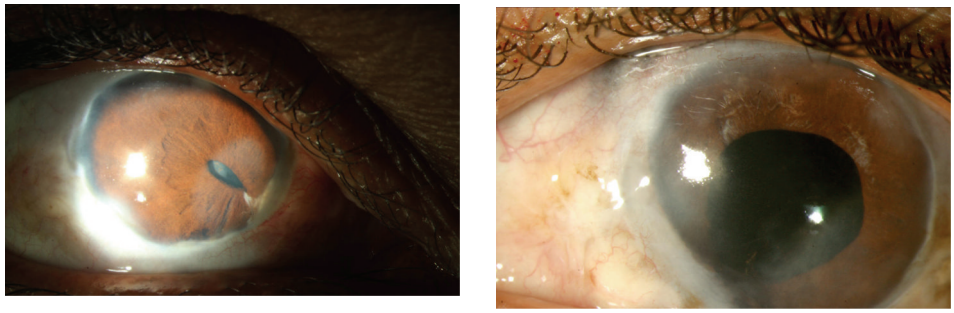
Figure 1: A 54-year-old farmer gave history of loss of vision and repeated episodes of redness and pain in the left eye following injury 40 years ago. B scan of the left eye showed findings suggestive of old vitreous hemorrhage and retinal detachment. Since visual acuity was doubtful perception of light, surgery was not advised for him. Diffuse (a) and slit (b) photographs show the left eye with peripheral corneal scar and peripheral anterior synechiae and total posterior synechia with cataract. At the temporal periphery, there is an incidental conjunctival lesion suggestive of actinic keratosis. Note the polychromatic crystals (cholesterolosis) deposited on the iris.

especially those with anterior uveitis in the pediatric age group [56] have more poor outcome than patients with ankylosing spondylitis and anterior uveitis.

The visual potential of the eye, determined by prior permanent structure damage, should be carefully determined before planning cataract surgery as this will have a direct impact on the visual outcome. The state of the macula and optic nerve should be thoroughly examined for during the preoperative assessment. Macular ischemia, atrophy, chronic macular edema, or scar, such as that resulting from a choroidal neovascular membrane, are poor prognostic factors. Similarly, optic atrophy and severe cupping of the optic disc are bad prognostic signs. Furthermore, the state of the retina must also be carefully examined for evidence of ischemia. In presence of dense lens opacity, B scan ultrasonography should be done to rule out retinal detachment which may complicate the eye with uveitis. In eyes with chronic retinal complications, possibly the cataract surgery may not result in optimal and desirable visual outcome and such cases are left to the discretion of surgeon to operate under nil visual prognosis or for cosmetic reasons (Figures 1(a) and 1(b)). Less disabling abnormalities such as pre-existing corneal scars and severe iris atrophy may also compromise the visual outcome.

Regardless of the guarded prognosis, a definite indication for cataract removal in an eye that is not blind, is phacoantigenic uveitis. This may result from the hypermature state of a cataract, whereby lens proteins leak out of the capsular bag through an intact capsule, or in cases of trauma, where the lens capsule has been breached, leading to persistence of intraocular inflammation. Cataract surgery may also be indicated to permit better visualization of the posterior segment for appropriate medical or surgical management of the eye [57].

4.2. Control of Pre Operative Inflammation. The risk of reactivation of uveitis must be assessed. Jancevski and Foster recommended the use of supplementary perioperative anti-inflammatory therapy to prevent damage to ocular structures essential to good vision [58]. This has been shown to reduce the risk of postoperative CME [59]. In eyes which are at risk of developing macular edema postoperatively, such as chronic anterior uveitis secondary to sarcoidosis; or eyes with previous episodes of CME (e.g., intermediate uveitis), steroid prophylaxis should be given perioperatively to protect against recurrence of macular swelling. Similarly, steroid prophylaxis should be administered in eyes at risk of developing recurrence of uveitis following cataract surgery, for example, Vogt-Koyanagi Harada disease, Behcet's disease and birdshot choroidopathy, to name a few. This may take the form of oral steroids 1 mg per kg/day starting 3 days preoperatively, tapering the steroid dose according to the amount of inflammation postoperatively. Generally, the oral steroids are tapered or reduced to the preoperative levels over the subsequent month, whilst maintaining the dose of other concurrent immunosuppressive therapy. Alternatively, if there are no contraindications to periocular steroid injections, such as documented steroid response or infectious uveitis, an orbital floor or sub-tenon's injection of depot steroid, such as triamcinolone acetonide 40 mg/1 mL may be given, especially in patients where high doses of oral steroid are contraindicated, for example in poorly controlled diabetics. In addition, guttae prednisolone acetate 1% 2 hourly administered 2 days prior to surgery, together with an oral and topical non-steroidal anti-inflammatory agent, can be given. Supported by encouraging results in the recent literature, the authors favour an intravitreal injection of preservative-free triamcinolone acetonide 4 mg in 0.1 mL at the conclusion of cataract surgery [60–62]. This has been shown to be as effective as prescribing systemic steroids perioperatively. Similarly, optimal control of periocular inflammation is imperative in cases with sclerokeratouveitis for optimal surgical and visual outcome (Figures 2(a) and 2(b)). In eyes with types of infectious uveitis that have a propensity to recur, such as ocular toxoplasmosis and herpes simplex uveitis, preoperative prophylaxis should also be considered as surgery may trigger reactivation of the infection. Toxoplasmic retinochoroiditis is associated with a 36% risk of reactivation following surgery [63]. Herpes



(a) (b)

Figure 2: A 48-year-old lady was diagnosed as bilateral sclera-keratouveitis, with complicated cataract. She was investigated extensively and had a positive Mantoux test, for which she received anti-tubercular therapy. She underwent cataract surgery in the left eye with a preoperative visual acuity of counting fingers. Postoperatively, her visual acuity improved to 20/125, limited by the presence of a central corneal scar. (a) Shows the right eye showing evidence of healed scleritis, corectopia, and a complicated cataract. (b) Shows the left eye two months postoperatively.

simplex is also associated with reactivation following the stress of surgery, and acyclovir 400 mg bid or valtrex 0.5 g qd preoperatively and for 2 to 3 weeks postoperatively may help prevent recurrence. In addition, topical NSAID and prednisolone acetate 1% or even oral NSAID may help control postoperative inflammation [64].

4.3. Complications of Uveitis Adversely Affecting Surgical Outcome: High-Risk Surgical Cases. Determining the surgical risk is a very important aspect of preoperative assessment. Eyes which have generally low intraocular pressures, especially readings of 6 mmHg or less even when quiescent, are at high risk of developing postoperative hypotony or even phthisis bulbi. Other warning signs such as seclusio papillae with normal intraocular pressure reading and apparent phacodonesis without evident zonulysis are important poor prognostic signs for postoperative hypotony. Eyes in which the uveitis is difficult to control are also at high risk of severe postoperative inflammation and hypotony or phthisis bulbi. The presence of choroidal effusion on B scan ultrasonography or diffusely thickened choroid is a poor prognostic sign. Conducting a careful ultrasonic biomicroscopy is essential in eyes with relative hypotony [65] to assess the state of the ciliary body and its processes. If the ciliary body has undergone atrophy, the risk of hypotony is high. If the ciliary body is found to be detached and processes appear under traction from a ciliary (cyclitic) membrane, cataract surgery should be combined with vitrectomy and trimming of the ciliary membrane aided by indentation of sclera to relieve ciliary body traction and to restore normal IOP.

4.4. Diagnostic Aids. Apart from the standard means of assessing macular, optic nerve and retinal function, one may apply additional methods such as pupillary response, light projection, colour perception and B scan ultrasonography, or an attempted OCT in looking for macular atrophy, edema, or hole [66]. Performing a macular potential test using laser interferometry may help in determining minimum visual potential [67]. A fundus fluorescein angiogram may also demonstrate macular ischemia or edema, retinal ischemia, active posterior segment disease, including disc leakage [68]. Considering the risk of post operative hypotony and to rule out cyclitic membrane preoperatively in chronic uveitic cases with bound pupils as described above, ultrasound biomicroscopy can also aid preoperative surgical planning [65]. Finally, the laser flare meter is a useful tool to measure flare in the anterior chamber and may be used to determine if the eye is quiet. It also helps guide therapy as it can be used to monitor the level of inflammation in the anterior chamber during the postoperative period [69].

4.5. Optimal Time for Cataract Surgery. Before scheduling surgery, the ophthalmologist should attempt to ensure that the eye has been quiescent for at least 3 months [11]. This has been shown to reduce the risk of postoperative CME [59]. In cases where, despite heavy immunosuppression, the intraocular inflammation is still not completely abolished and surgery is urgently required, such as in an intumescent cataract, the patient may be administered intravenous methylprednisolone 1 g one day before surgery. A study from Japan suggests that in patients with Behcet's disease the eye should be inactive for a minimum of 6 months and that the risk is higher if attacks have occurred within 12 months of cataract surgery [70].

4.6. Counselling of the Patient for Uveitic Cataract Surgery. The most important aspect of counselling when planning to perform cataract surgery for the uveitic eye is explaining the visual prognosis. The general risks involved in surgery, such as infection and other intraoperative complications will also need to be thoroughly explained, especially if there is phacodonesis, hypotony, or glaucoma. Emphasizing that the eye will need a minimum period of quiescence before surgery to minimise the chance of recurrence and improve the visual outcome is important. Furthermore, it is important to explain that surgery may be complicated and possibly take longer than usual because of abnormal anatomy, such as the presence of synechiae, membranes,

and so forth, and that these factors may contribute to postoperative inflammation. Other factors requiring careful discussion and explanation include the possibility of and reasons for delayed visual recovery, the need for compliance with medications (systemic immunosuppression may need to be adjusted), and frequent followup, especially if the patient has difficulty accessing medical care.

These patients are often young, therefore losing a lens that was still able to accommodate in exchange for an intraocular lens implant means loss of accommodation. Consequently, they will need to understand and accept the fact that they will now need reading glasses. The type of IOL implant, the material, and design are all important points that need discussion. The choice of intraocular lens can be based on the extensive literature available [39, 71–74].

Generally, multifocal implants, whether based on diffractive or refractive principles may compromise the visual outcomes due to the presence of preexisting macular or optic nerve conditions. Hazy or scarred vitreous gel contributes to poor contrast sensitivity and any previous episode of inflammation with macular involvement increases the risk of poor visual performance with multifocal implants. These patients do better with a monofocal IOL implant. Even accommodative IOLs may not be effective in the long term due to recurrent inflammation and scarring of the ciliary body, or slowly progressive fibrosis of the capsular bag.

Posterior capsule opacification is another frequent complication encountered postoperatively because of the relative youth of these patients [75]. Choice of IOL, as will be discussed later (see IOL implantation-contraindications and type of IOL), and surgical technique are major determining factors as well. Occasionally, opacities are observed during the surgery, and some surgeons prefer to perform a primary posterior capsulorhexis at the time of the cataract surgery before implanting the IOL [76]. This possibility should be discussed with the patient before surgery, especially in view of the increased risk of postoperative endophthalmitis, CME, and retinal detachment.

Should the patient have complications other than cataract formation alone, the option of separate, staged, or combined surgery should be discussed with the patient and advice given regarding their risks and benefits [77].

5. Surgical Technique

5.1. Choice of Surgery. The choice of cataract surgery technique is best left to the surgeon and depends upon the individual surgeon's surgical skill and experience. Cataract removal by phacoemulsification is safer for the uveitic cataract as less inflammation is induced than that by a manual extracapsular cataract extraction. During the surgery, the anatomy of the anterior segment should be restored to a state as close to normal as possible.

Some uveitic cataract eyes are complicated by glaucoma or retinal problems that may also benefit from surgery. For eyes with concomitant uveitic glaucoma, surgery is preferably not combined with the cataract surgery as the risk of bleb failure is increased with drainage of post-cataract surgery inflammatory exudate through a healing bleb. Where possible, cataract surgery should be done first. Regarding retinal complications, such as epiretinal membranes or coexisting retinal detachment, cataract surgery may be combined with vitreoretinal surgery. In cases with major retinal problems, the eye may be safely rendered aphakic until the retinal problem has been dealt with. In eyes with intermediate uveitis, or FHI, cataract surgery may be combined with vitrectomy, performed to clear the vitreous gel, thereby reducing vitreous clouding. In intermediate uveitis, this often not only improves vision but also controls intraocular inflammation and helps resolve the cystoid macular edema. At the end of surgery, especially with combined surgical procedures, having excluded steroid responders and eyes with infectious uveitis, an intravitreal injection of triamcinolone acetonide, is often adequate to control the postoperative inflammation and prevent CME. The risks and benefits of combining or separating the surgical procedures should be thoroughly explained to the patient.

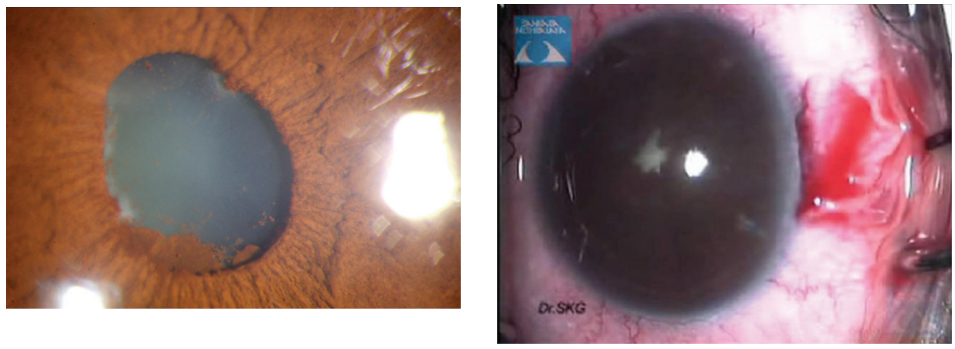
5.2. Intraoperative Surgical Techniques and Skills [78]

(a) Posture. Patients with ankylosing spondylitis with a fixed flexion deformity of the axial spine, especially when the cervical spine is involved, have difficulty not only in placing their chin on the slit-lamp rest but also lying flat on the operating table for ocular surgery. These patients are best postured in the Trendelenburg position, whereby their lower limbs are elevated above the level of their head, so as to maintain the plane of the face parallel to the floor. The pillow support may need to be stacked up high in order to support the head. As the patient tends to slide down the bed, a strap is best secured around the torso to prevent the body from slipping.

(b) Surgical Challenges. The uveitic eye poses numerous surgical challenges. These include the small pupil, shallow anterior chamber, posterior synechiae, peripheral anterior synechiae, pupillary membranes and even zonulolysis. Complications that may arise from the problems include an undersized or incomplete capsulorhexis, iris prolapse, increased risk of posterior capsular rent, increased risk of intraoperative zonular dehiscence, and increased postoperative inflammation.

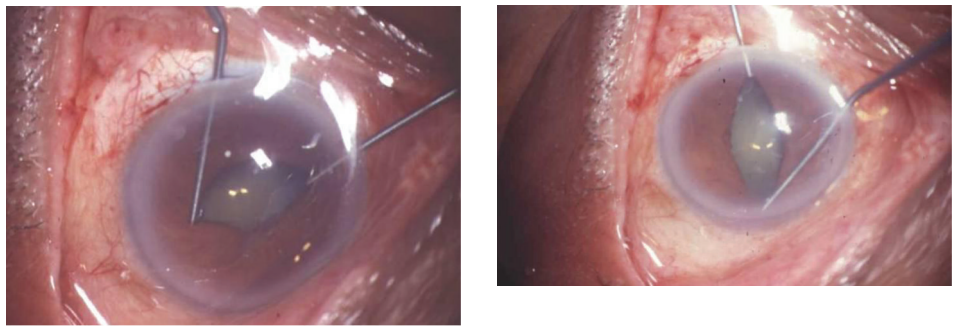
(c) Anaesthesia. Whilst phacoemulsification surgery may be done under topical anesthesia, manipulation of the iris may induce ocular discomfort or pain. Either regional anesthesia or an intracameral injection of preservative-free lignocaine 1% can provide adequate analgesia. For children and in patients for whom prolonged surgical time is anticipated, as in severe zonulolysis requiring modified capsular tension rings that need suturing, general anesthesia may be preferred.

(d) Incision. Either a scleral or temporal clear corneal incision may be used. However, the incision should be of



(a) (b)

Figure 3: (a) Showing the eye with synechiae at papillary border, pigment deposition on the lens, and an early cataract and (b) showing presence of 360 degree posterior synechiae.



(a) (b)

Figure 4: Intraoperative use of Kuglen hooks to stretch and dilate the pupils.

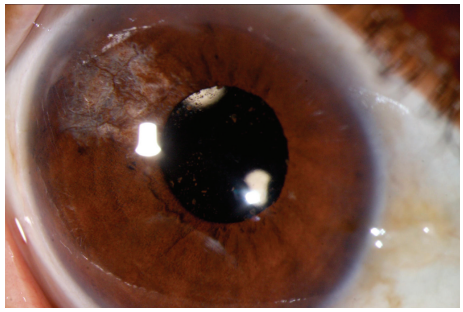
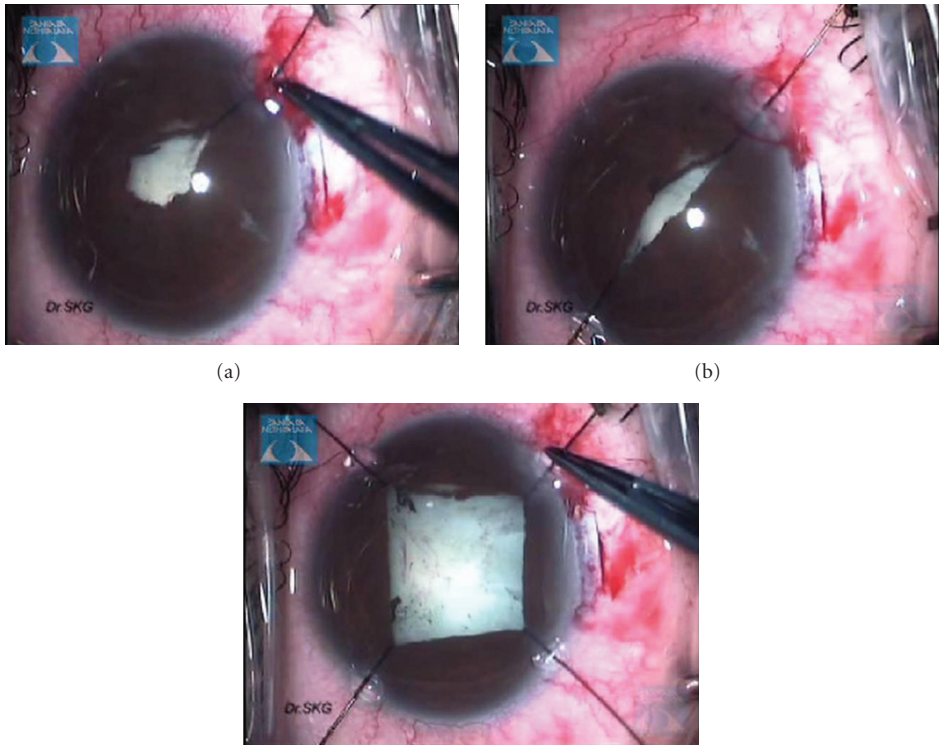


Figure 5: Postoperative slit lamp photograph showing minimally distorted pupil following pupil manipulation intraoperatively to negotiate posterior synechiae.

adequate length in order to prevent iris prolapse in eyes with small or stretched pupils.

(e) Pupil Enlargement. An attempt at pupil dilation can be made by injecting balanced salt solution with adrenaline (1 : 1000 0.5 mL adrenaline in 500 mL) into eyes with pupils that are not bound by synechiae or membranes. Preservative-free intracameral lignocaine 1% may also be used to help dilate the pupil only if it is not bound. Choosing a viscoadaptive viscoelastic such as Healon 5 (sodium hyaluronate 2.3%, Abbott Medical Optics) is useful as this high-molecular-weight sodium hyaluronate can physically roll open the pupil and keep it dilated as long as the aspiration flow rate is kept low.

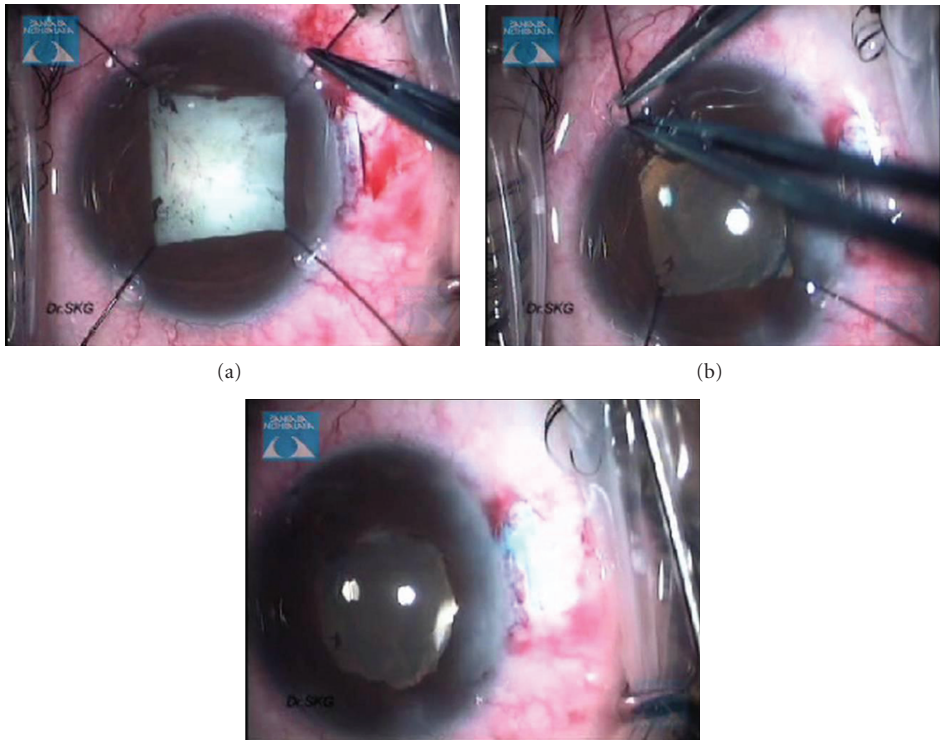
(f) Synechiolysis and Removal of Pupillary Membrane. Synechiae may be present between the iris and the anterior lens capsule (posterior synechiae, PS) (Figures 3(a) and 3(b)) or may form between the peripheral iris and corneal endothelium as a result of previous iris bombe (peripheral anterior synechiae, PAS). When both are present, the PAS should be released before the PS. Release of PAS may be done by injecting viscoelastic, such as Healon 5 (Viscoadaptive from Abbott Medical Optics, Inc. Abbott Park, Ill, USA), to physically separate the iris from the cornea, failing which the tip of the viscoelastic cannula may be used to sweep the iris away from the peripheral cornea as the viscoelastic material is being injected into the angle of the anterior chamber. This should be done very gently and carefully, taking care not to detach Descemet's membrane in the process. PS may be lysed by simply injecting viscoelastic against the adherent iris, allowing the viscoelastic to "bulldoze" the iris away from the anterior capsule. Alternatively, this may be done by



(a) (b)

(c)

Figure 6: Intraoperative use of self-retaining iris hooks to dilate the pupils.



(a) (b)

(c)

Figure 7: Postoperatively removal of self-retaining iris hooks.

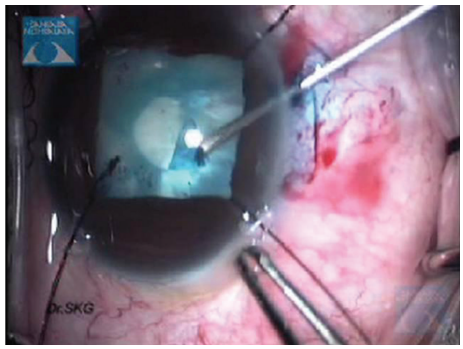


Figure 8: Intraoperative still video clip demonstrating the step of capsulorrhexis.

sweeping the pupil free from the lens capsule with a cannula. In cases where a narrow strip of membrane is present at the site of PS formation, a 27-gauge needle may be used to simultaneously nick the membrane to segment and release it from the anterior capsule and release the PS, thus stretching the pupil.

(g) Pupil Expansion. The most user-friendly instrument for extensive synechiae once an edge of the iris has been viscodissected off the anterior capsule is a bent Kuglen hook. This "push-pull" instrument is excellent for the safe release of PS, ranging from mild to extensive, as it enables the surgeon to push or pull the iris, thereby releasing the iris from the anterior lens capsule, even when a pupillary membrane is present. When the pupil has been freed, the membrane can be removed using a pair Kelman-Mcpherson forceps. Often, once the pupillary membrane has been removed, the pupil begins to widen with viscoelastic. However, if this is inadequate, the pupil may be stretched using a pair of angled Kuglen hooks introduced through the main incision, used in a manner to latch around the pupil edge, pulling the iris in opposite directions (Figure 4(a)). This is then repeated in a direction perpendicular to the initial stretch (Figure 4(b)). The surgeon should stop at once should the iris sphincter develop a tear. If proper execution of pupil manoeuvring is done, postoperatively pupil looks relatively round with minimal distortion of pupillary margins (Figure 5). The pupil may then be further enlarged using multiple sphincterotomies by means of intraocular scissors, taking care not to compromise the anterior capsule.

Alternative means of opening the pupil include the use of a Beehler pupil dilator (2 or 3 pronged) to mechanically stretch the pupil in a single injector system. Pupil retainers may also be tried. Disposable iris hooks are easy to place through multiple corneal paracentesis (Figures 6(a)–6(c)). The iris hooks are removed at end of surgery (Figures 7(a)–7(c))). More recently a pupil device, the Malyugin ring (Microsurgical Technologies, Redmond, Wash, USA) has been used, which may be injected into the anterior chamber through a 2.2 mm incision and manoeuvred to expand and maintain the pupil open at a 6 or 7 mm diameter.

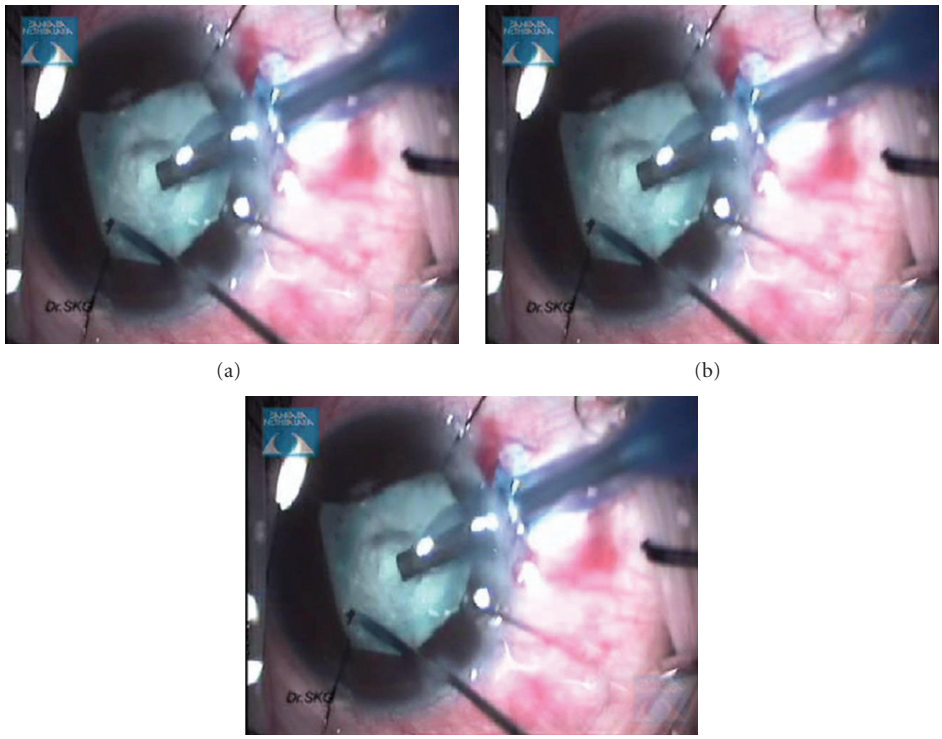
(h) Continuous Circular Capsulorhexis. It is generally preferable to keep the size of the capsulorhexis slightly smaller than the pupil so that iris chaffing does not occur and results in progressive intraoperative miosis as nuclear fragments are being moved out of the capsular bag (Figure 8). This also contributes to increased postoperative inflammation. When the pupil size is small, the capsulorhexis inevitably needs to be larger than the pupil. The anterior chamber must be kept deep and the anterior capsule flattened using adequate viscoelastic material in order to control the tearing of a capsulorhexis. The capsulotomy can be initiated by a 26- or 27-gauge bent cystitome and a modified vitreoretinal forceps (pediatric rhexis forceps) can be then inserted from the side port to complete the rhexis. Creating the ideal capsulorhexis is also very important in preventing posterior capsule opacification. The capsulorhexis should be centred, overlapping the edge of the optic at all times, but not so small as to prevent capsular phimosis.

(i) Nucleus Management. In small pupils, the safest technique is the vertical chop employed in the in situ chop technique. Chopping of fragments is done within the pupillary aperture with the phaco tip kept in view at all times with minimal risk of engaging and traumatizing the iris (Figures 9(a) and 9(b)).

(j) Irrigation and Aspiration. This step must be done thoroughly so as not to leave cortical material behind. The eye should be rotated to look for residual lens matter and a gentle shake given at the end of nucleus removal to ensure that no fragments are still lodged in the posterior chamber during phaco (Figure 10).

(k) Intraocular Lens Implantation Contraindications and Type of IOL. The review of the literature suggests that while earlier, implantation of IOLs in uveitic eyes with JIA and chronic uveitis was considered a contraindication, now with modern IOLs, it may be safe to implant IOLs in these difficult case scenarios as long as the uveitis is well under control. Alió et al. [39, 79] showed that the most biocompatible IOL for the anterior chamber and the capsular bag is a single-piece, square-edged acrylic (either hydrophilic or hydrophobic) IOL. They also found that the posterior capsular opacification rate was highest (34.2%) in eyes with silicone IOLs. In addition, silicone IOLs had a higher incidence of postoperative cystoid macular edema and PS formation, and pupillary membranes were formed only in eyes with silicone IOLs. In general, hydrophilic acrylic material has good uveal but worse capsular biocompatibility, but hydrophobic acrylic material had lower uveal but better capsular biocompatibility [78]. In eyes with chronic uncontrolled uveitis, IOL implant should be deferred.

Removal of viscoelastic from under the IOL is an important step in reducing the space behind the IOL into which lens epithelial cells tend to migrate, thus causing PCO. Pressing the optic against the posterior capsule when using a single-piece hydrophobic IOL also encourages its adhesion to the posterior capsule, thereby reducing the risk of PCO [79].



(a) (b)

(c)

Figure 9: Intraoperative still surgical video clip showing the nucleus management in total white uveitic cataract.

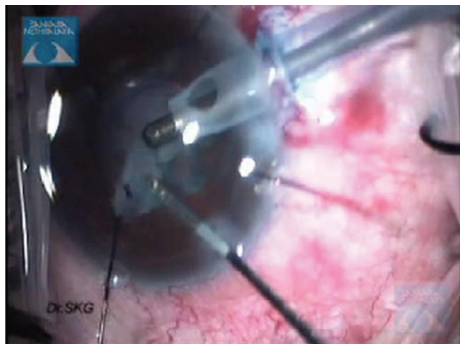


Figure 10: Intraoperative still surgical video clip showing irrigation and aspiration of the soft lens matter.

5.3. Complications and Postoperative Management

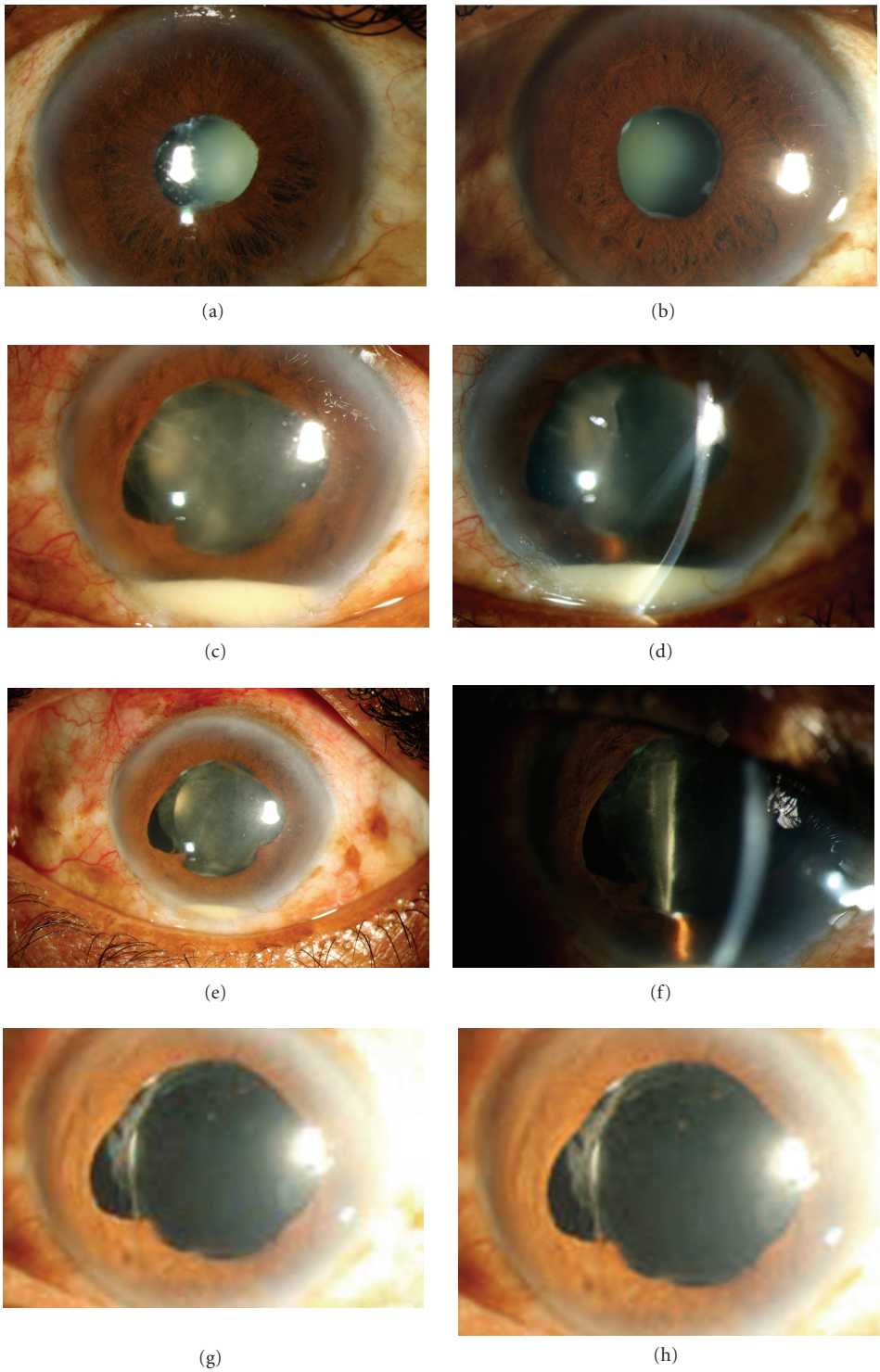
5.3.1. Intraoperative Complications

Zonulolysis. An infrequent intraoperative complication is Zonulolysis. This can occur in eyes with chronic uveitis. Insertion of a plain capsular tension ring (CTR) is often necessary in order to prevent IOL decentration. However, if the overall zonular strength is weak, fixation of the CTR to the sclera by means of a modified Cionni CTR ensures that the IOL remains centred. Failure to use a CTR in the presence of weak zonules may result in capsular phimosis, due to unopposed capsular bag fibrosis and shrinkage.

Retained Lens or Nuclear Fragments. Due to the small pupil size, small hard nuclear fragments may lodge in the posterior chamber during phacoemulsification, only to pop into the anterior chamber months later. These small fragments can cause recurrent anterior uveitis when their position in the anterior chamber changes and may also cause localized corneal edema and even localized corneal decompensation in the long term. Hence, at the end of phaco, the eye should be given a gentle shake whilst aspirating to ensure no nuclear fragments are inadvertently left behind. Any retained soft lens material or nuclear fragment should be removed surgically as soon as possible [80].

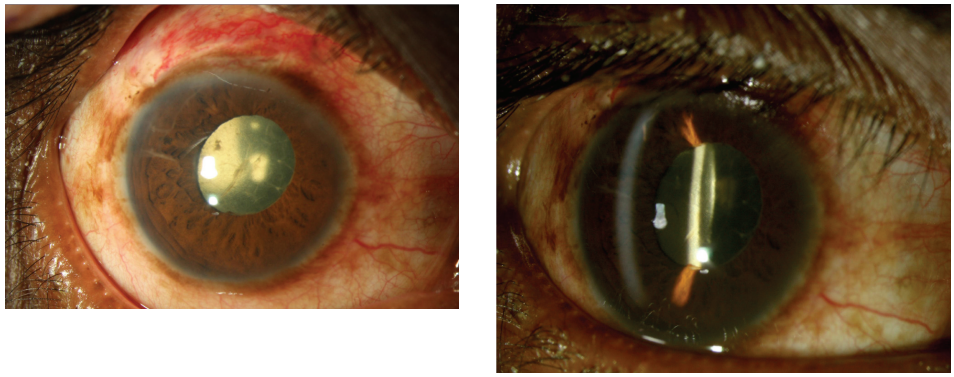
5.3.2. Early Postoperative Complications

Excessive Postoperative Inflammation. One of the most common postoperative complications is excessive postoperative inflammation (Figures 11(a)–11(g)). This may vary in terms of severity or duration of inflammation. Associated with this is the development of cystoid macular edema, which may be treated by controlling the inflammation. The incidence of CME following extracapsular cataract surgery in uveitic eyes is frequent and has been reported to range from 33% to 56% [10, 14]. Following phacoemulsification, the incidence has been reported to range from 12% to 59% [38]. Generally, if preoperative prophylactic oral steroids have been given and maximal topical steroids and cycloplegics have proven ineffective in controlling the uveitis, the dose



(a) (b) (c) (d) (e) (f) (g) (h)

Figure 11: A 45-year-old man, a treated case of Hansen's disease 15 years ago, presented with progressive visual loss. Examination showed active anterior uveitis and complicated cataract. He was treated with topical steroids and after 4 months, underwent phacoemulsification with PCIOL in the left eye. On the first postoperative day, his visual acuity improved to 20/50 (from preoperative vision of 20/200) with a mild fibrinous reaction in the anterior chamber. He returned to the emergency clinic on the next day with loss of vision and showed severe anterior chamber reaction with hypopyon. Vitreous appeared uninvolved. He was hospitalized and treated with intensive topical steroids and cycloplegics. He improved over the course of one week and regained good vision at the end of one month. (a, b) show the right and left eye with quiet anterior chambers and nondilating pupil with posterior synechia. (c, d) Diffuse and slit view of the left eye on the second postoperative day shows hypopyon and coagulum around the IOL. (e, f, g) Diffuse low and high magnification and slit view of the left eye two days after intensive treatment showing decrease in the inflammation. (h) The left eye shows near-quiet anterior chamber 2 weeks after treatment, the visual acuity has improved to 20/50.



(a) (b)

Figure 12: A 36-year-old man presented with a history of redness and pain since two years and decreased vision since one year. Examination showed a total cataract in the right eye, with 360 degrees posterior synechiae. Investigations showed HLA-B 27 was positive. After the inflammation subsided, the patient underwent cataract surgery with synechiolysis. Postoperatively, there was increased anterior chamber inflammation, which was treated with oral and topical steroids. The patient regained visual acuity of 20/25; 3 months postoperatively and is on maintenance with oral methotrexate and topical steroids. (a, b) Diffuse and slit photograph of the right eye on the first postoperative day shows a membrane on the IOL which responded to intensive topical steroids and cycloplegics.

of oral steroids may be sharply increased. If no prophylactic oral steroids had been given the patient should be given an oral pulse of steroids or injection of periocular steroids. An alternative means would be to give an intravitreal injection of triamcinolone acetonide [81] if this had not been given intraoperatively, thus avoiding the need to adjust the systemic immunosuppression. In the pediatric eye with chronic uveitis undergoing cataract surgery, a multistage surgery may be the safer approach, whereby various complications are addressed at different sittings [79]. This strategy may avoid postoperative complications and improve surgical outcomes.

Posterior synechiae, pupillary or ciliary membrane formation may occur during the postoperative period due to excessive inflammation (Figures 12(a) and 12(b)). Control of uveitis and keeping the pupil mobile during this time are important.

Intraocular Pressure (IOP) Abnormalities. The IOP may be raised transiently during the early postoperative period in eyes with compromised trabecular meshwork or angles. This can often be managed with topical and systemic antiglaucoma medications.

However, the surgeon's greatest fear is hypotony. Once wound leakage has been ruled out, the next step is to increase the anti-inflammatory therapy topically and systemically. This is often effective in raising the IOP, but the topical steroids may be difficult to taper or withdraw and patients may require long-term topical steroids to maintain the IOP. Stabilisation of the IOP and vision has been successfully treated with an intraocular injection of sodium hyaluronate via a limbal paracentesis in non uveitic eyes [82]. In severe cases, vitrectomy and trimming of ciliary body traction membranes and silicone oil filling may be needed if UBM shows the presence of ciliary body detachment secondary to tractional membranes not addressed during the cataract surgery [83].

Recurrence of Uveitis. Increased frequency of recurrence following cataract surgery may occur. This is thought to be triggered by the intraocular procedure. The recurrence rate has been reported to be as high as 51% [15]. Hence, stepping up the immunosuppression for the long term may be necessary to prevent further recurrences.

5.3.3. Late Postoperative Complications

Posterior Capsular Opacification. In the late postoperative period, posterior capsular opacification is perhaps the most common complication following any type of cataract surgery. Okhravi et al. [9] reported an incidence of 48.0%, Rauz et al. [84] 81.7% and Küçükerdönmez et al. [82] 34.2% at 1 year. Their corresponding Nd:YAG capsulotomy rates were 32.2%, 8.3%, and 3.6%.

Preventive measures include creating a circular well-centred capsulorhexis which is smaller than the optic size, using an acrylic IOL with a square-edged optic design, meticulous removal of viscoelastic from within the capsular bag and ensuring the optic is stuck on to the posterior capsule at the conclusion of surgery. Control of postoperative inflammation also plays an important role in preventing PCO.

Explanting an IOL. Removal of intraocular lens in uveitic eyes is rarely necessary. Foster et al. reported that their indications include the formation of perilental membrane, chronic low-grade inflammation not responding to anti-inflammatory treatment and cyclitic membrane resulting in hypotony and maculopathy [85, 86]. The underlying diagnosis for uveitis included sarcoidosis, JIA, and pars planitis, in eyes with predominantly intermediate or panuveitis, with the inflammation centered on the pars plana region. They believe that undetected subclinical inflammation present chronically after surgery was responsible for the postoperative

complications leading to IOL removal despite the necessary precautions having been taken during the perioperative period.

6. Conclusion and Summary

It is possible to achieve successful visual outcomes following cataract surgery in uveitis with the modern day cataract surgery. The predictability has improved mainly because of a higher level of understanding of the uveitic disease among clinicians. Preoperative factors include proper patient selection and counseling and preoperative control of inflammation. It is now well recognized that chronic inflammation, even low grade, can irreversibly damage the retina and optic nerve [6], and therefore control of inflammation, both pre- and postoperatively, is vital. The use of immunosuppressive agents other than steroids also helps control inflammation and has enabled long-term use of these agents especially as steroids sparing medication. Management of postoperative complications, especially inflammation and glaucoma, earlier rather than later, has also contributed to improved outcomes. Still several questions remain unanswered, especially in the area of pediatric uveitis with cataract, which continue to challenge the ophthalmologist to further refine the surgical technique and search for new treatment modalities [56, 57]. In conclusion, management of the uveitic cataract requires careful case selection, proper timing of surgery, meticulous surgery and close monitoring with appropriate handling of the postoperative complications that may occur. These eyes can achieve good outcomes with proper management.

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Clinical Study

Intravitreal Dexamethasone in the Management of Delayed-Onset Bleb-Associated Endophthalmitis

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Purpose. To report the visual acuity (VA) outcomes and culture results of delayed-onset bleb-associated endophthalmitis (BAE) with and without intravitreal dexamethasone (IVD). *Methods.* Retrospective nonrandomized comparative case series of BAE at Bascom Palmer Eye Institute between January 1, 1996 and December 31, 2009. Clinical data were compared using the 2-sided Student's *t*-test for patients who received IVD and patients who did not receive IVD. *Results.* 70/83 (84%) received IVD, and 13/83 (16%) did not receive IVD. Mean baseline VA was 20/90 in the IVD group and 20/70 in the group that did not receive IVD ($P = 0.57$). Mean presenting VA was 0.9/200 in the IVD group and 1.7/200 in the group that did not receive IVD ($P = 0.23$). Repeat cultures were positive in 2/70 (3%) IVD cases and 1/13 (8%) cases that did not receive IVD ($P = 0.57$). Mean VA at 1 month was 5/200 in the IVD group and 1.8/200 in the group that did not receive IVD, logMARΔ of 0.85 and 1.56, respectively ($P = 0.02$). Mean VA at 3 months was 7/200 in the IVD group and 3/200 in the group that did not receive IVD, logMARΔ of 0.74 and 1.33, respectively ($P = 0.14$). *Conclusion.* In the current study of BAE, IVD was associated with improved short-term VA outcomes without an increased rate of persistent infection.

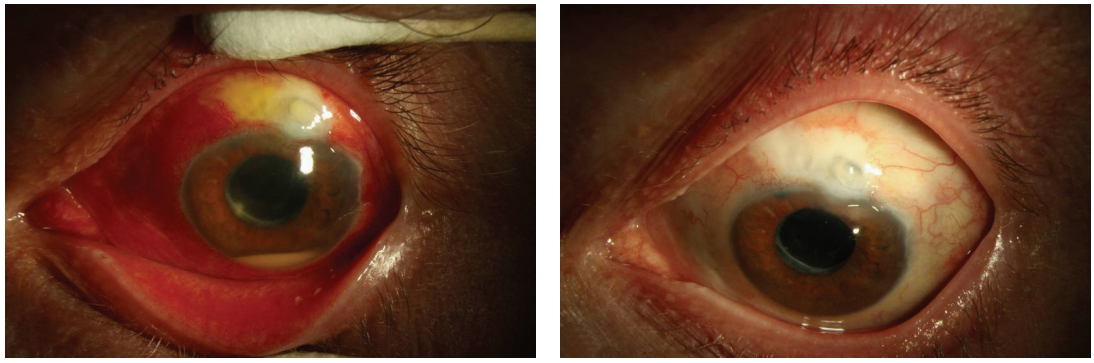
1. Introduction

Since 1974 intravitreal dexamethasone (IVD) has been used as an adjunct to intravitreal antibiotics in the management of bacterial endophthalmitis [1–3]. In 1992, Irvine et al. reported favorable outcomes in a series of Gram-negative endophthalmitis cases treated with adjunctive IVD [2]. In 2004 43% of retina specialists responded that they use IVD in the management of postcataract endophthalmitis [4]. The role of IVD in the management of bacterial endophthalmitis remains controversial due to contradictory results reported by small, comparative studies [5–8].

In delayed-onset bleb-associated endophthalmitis (BAE), the majority of reported cases, 53–82%, received adjunctive IVD [9–12]. No BAE series however has yet reported the VA outcomes of cases treated with and without IVD. Unlike postcataract endophthalmitis, BAE is commonly associated with virulent *Streptococcus* and Gram-negative organisms [10–13]. If IVD potentiates intraocular infection then VA outcomes may be worse in BAE cases treated with IVD. Additionally, the culture data of second biopsies in BAE cases treated with IVD may manifest a higher rate of persistent infection. The current study reports the VA outcomes and culture results of BAE cases treated with and without IVD to further clarify the role IVD plays in the management of bacterial endophthalmitis.

2. Methods

The study protocol was approved by the Institutional Review Board of the University of Miami Miller School of Medicine Subcommittee for the Protection of Human Subjects in Research. The medical records and microbiologic records of all patients treated for BAE at Bascom Palmer Eye Institute (BPEI) between January 1, 1996 and December 31, 2009 were reviewed. As a nonrandomized comparative case series the decision to use or not use IVD was made by the individual



(a) (b)

Figure 1: Photographs of the left eye of 55-year-old male presenting with BAE from *Moraxella*. (a) Presenting VA: HM, IOP: 19 mmHg. Treatment: pars plana vitrectomy with intravitreal Vancomycin, Ceftazidime, and Dexamethasone. (b) At 3 months VA: 20/40, IOP: 14 mmHg.

treating physician and did not involve a prospective protocol. All patients had prior glaucoma filtering surgery. BAE was defined as intraocular infection with vitreous involvement receiving treatment with intravitreal antibiotics. Patients with tube shunts as the filtering mechanism, bleb infection only (no posterior inflammation), onset within 1 month of glaucoma surgery, and inadvertent filtering blebs after cataract surgery were excluded. Clinical history and presentation, treatment, intraocular culture data, VA outcomes, and factors affecting VA were recorded. The current study included clinical information from the BPEI series of BAE previously published [9, 13].

Snellen VAs were converted to logMAR equivalents for statistical analysis; VAs of HM, LP, and NLP were assigned logMAR values of 2.6, 3, and 4 respectively. Change in VA was determined by comparing the last recorded VA before the onset of endophthalmitis (pre-endophthalmitis VA) with VA at 1 and 3 months. Three or more lines of improvement ($\geq$3 lines improvement) were determined by comparing the VA at presentation of endophthalmitis (presentation VA) with the VA at 1 and 3 months. The mean logMAR change after presentation (logMARΔ) and other clinical data were grouped according to IVD use and compared using the 2-sided Student's t-test. Logistic regression was used to determine the odds ratio for IVD as a predictive factor for $\geq$3 lines improvement. A P value of $\leq$0.05 was considered statistically significant.

3. Results

In the current study, 86 eyes were identified. Excluded were 1 eye with a preexisting tube shunt and 2 eyes that underwent primary evisceration. Of the 83 eyes, 70 (84%) received IVD and 13 (16%) did not receive IVD. None of the patients received systemic steroid. In all cases, the causative organisms were sensitive to the intravitreal antibiotics clinically administered. Baseline demographics, clinical presentation, and initial culture results were similar between the two groups with a few exceptions (Table 1).

A greater percentage of IVD cases presented with poor view of the fundus, 69% compared to 39%. The majority of both groups received an initial treatment of tap and injection (T&I); however, a higher percentage received pars plana vitrectomy (PPV) in the IVD group, 41% compared to 8%. Also a higher percentage of IVD cases were culture-positive, 66% compared to 46%, but this difference did not reach significance.

Repeat cultures were performed during a second procedure in 11/70 (16%) IVD cases and 3/13 (23%) of cases that did not receive IVD (Table 2).There was no significant difference in primary or repeat culture-positive results between the two groups. The repeat culture-positive rate was 2/70 (3%) for IVD cases and 1/13 (8%) for the cases that did not receive IVD. In each of these 3 cases the same causative organism was isolated in the repeat culture as in the initial culture. Repeat cultures were positive in 1/21 (5%) *Streptococcus* and 2/6 (33%) *Enterococcus* cases.

IVD cases had worse mean pre-endophthalmitis and presentation VA but this did not reach significance (Table 3).

At 1 month mean VA was 5/200 in the IVD group and 1.8/200 in the group that did not receive IVD, logMARΔ of 0.85 and 1.56, respectively ($P = 0.02$). At 3 months mean VA was 7/200 in the IVD group and 3/200 in the group that did not receive IVD, logMARΔ of 0.74 and 1.33, respectively ($P = 0.14$). A higher percentage of IVD cases achieved $\geq$3 lines improvement at 1 and 3 months. Logistic regression showed that IVD was a significant predictive factor of $\geq$3 lines improvement at both 1 and 3 months (Table 4).

4. Discussion

Corticosteroids are often used as an important adjunct to antibiotics and PPV in the management of infectious bacterial endophthalmitis (Figure 1). Corticosteroids are known to reduce the degree of inflammation caused by toxins liberated from microorganisms. The role of IVD in the management of postcataract bacterial endophthalmitis is unclear due to contradictory results of small, comparative studies (Table 5).

Table 1: Baseline demographics, clinical presentation, and initial culture results.

	IVD 70/83 (84%)	No IVD 13/83 (16%)	*P* value
Age			
Mean, SD	74 yr (12)	70 yr (14)	0.27
Gender			
Female	34 (49%)	8 (62%)	0.39
Male	36 (51%)	5 (39%)	
Diabetes mellitus			
Present	9 (13%)	2 (15%)	0.81
Absent	61 (87%)	11 (85%)	
Antimetabolites (MMC or 5FU)			
Used	45 (64%)	7 (54%)	0.47
Not used	25 (36%)	6 (46%)	
Mean time of onset, SD	60 mo (43)	49 mo (55)	0.46
Bleb leak			
Present	16 (23%)	5 (38%)	0.23
Absent	54 (77%)	8 (62%)	
Anterior chamber			
Hypopyon	48 (69%)	10 (77%)	0.55
View to fundus			
Hazy	22 (31%)	8 (62%)	0.04
Poor/none	48 (69%)	5 (39%)	
Intraocular Pressure			
Presentation, SD	20 (14)	19 (12)	0.8
Treatment, initial			
Tap and injection	41 (59%)	12 (92%)	0.03
Pars plana vitrectomy	29 (41%)	1 (8%)	
Treatment, additional			
Filtering procedure	12 (17%)	1 (8%)	0.39
Pars plana vitrectomy	21 (30%)	2 (15%)	0.28
Culture results			
Culture positive	46 (66%)	6 (46%)	0.18
Culture negative	24 (34%)	7 (54%)	
Gram-positive cases	33 (47%)	4 (31%)	0.28
Streptococcus	19 (27%)	2 (15%)	0.37
Coagulase-negative *Staphylococcus*	7 (10%)	2 (15%)	0.57
Enterococcus	6 (9%)	0	0.27
Staphylococcus aureus	1 (1%)	0	0.67
Gram-negative cases	12 (17%)	2 (15%)	0.88
Moraxella	8 (11%)	0	0.2
Pseudomonas	2 (3%)	1 (8%)	0.39
Serratia	1 (1%)	1 (8%)	0.18

Table 2: Repeat culture results.

	IVD 70/83 (84%)	No IVD 13/83 (16%)	*P* value
Repeat Cultures Performed:			
Number of eyes	11 (16%)	3 (23%)	0.52
Mean time, days (range)	20 (1–60)	14 (2–30)	0.64
Primary culture results			
Streptococcus	4 (36%)	2 (67%)	
Enterococcus faecalis	2 (18%)	0	
Coagulase-neg *Staph.*	2 (18%)	1 (33%)	
Enterobacter aerogenes	1 (9%)	0	
No growth	2 (18%)	0	0.43
Repeat culture results			
Streptococcus	0	1 (33%)	
Enterococcus faecalis	2 (18%)	0	
Coagulase-neg *Staph.*	0	0	
Enterobacter aerogenes	0	0	
No growth	9 (82%)	2 (67%)	0.57
Repeat culture positive rate	2 (3%)	1 (8%)	0.57

Table 3: VA outcomes.

	IVD 71/84 (84%)	No IVD 13/84 (16%)	*P* value
VA, pre-endophthalmitis	$n = 67$	$n = 13$	
Mean	20/90	20/70	0.57
Range	20/20-LP	20/25–20/400	
VA, presentation	$n = 70$	$n = 13$	
Mean	0.9/200	1.7/200	0.23
Range	20/40-NLP	20/80-LP	
VA, 1 month	$n = 66$	$n = 12$	
Mean	5/200	1.8/200	0.14
Range	20/25-NLP	20/25-NLP	
≥3 lines Improvement	44 (67%)	3 (25%)	0.01
logMARΔ	0.85	1.56	0.02
VA, 3 months	$n = 56$	$n = 9$	
Mean	7/200	3/200	0.36
Range	20/25-NLP	20/25-LP	
≥3 lines Improvement	36 (64%)	3 (33%)	0.14
logMARΔ	0.74	1.33	0.14

Table 4: Predictive factor ≥3 lines improvement.

IVD versus No IVD	Odds ratio, (CI)	*P* value
1 month	7.04 (1.63,30.43)	0.01
3 months	5.21 (1.07,25.37)	0.04

Das et al. reported favorable results of reduced intraocular inflammation at 1 and 4 weeks in eyes treated with IVD [5]. Gan et al. additionally found a trend toward better visual outcomes at 3 and 12 months in eyes treated with IVD [6]. In contrast, Hall et al. reported no difference in inflammation and VA outcomes at last followup in eyes treated with IVD [7]. Shah et al. found worse VA outcomes at 1, 3, and 6

Table 5: Comparative studies of IVD for bacterial endophthalmitis.

	Clinical setting	Culture results			Inflammation	VA outcomes	Time
	n	*Staph epi.*	*Strep/Enterococcus*	Gram-negative			
Das et al. [5]	Postcataract and trauma						
IVD	29	n/a	n/a	n/a	2.6 score	86% success	3 months
No IVD	34				3.2 score[1]	71% success	
Shah et al. [8]	Postcataract						
IVD	26	31%	12%	0	n/a	20/70 median	6 months
No IVD	31	35%	13%	3%		20/50 median[2]	
Gan et al. [6]	Postcataract						
IVD	16	39%	8%	0	n/a	85% 20/200 or better	3 months
No IVD	13	50%	6%	0		50% 20/200 or better[3]	
Hall et al. [7]	Postcataract						
IVD	26	46%	23%	0	0.3 cell/flare	20/40 median	last followup
No IVD	38	37%	5%	0	0.3 cell/flare	20/50 median	
Jacobs et al.	Bleb-associated						
IVD	70	10%	36%	17%	n/a	7/200 mean	3 months
No IVD	13	15%	15%	15%		3/200 mean[4]	

[1]Relative change in inflammation showed statistical significance at 1 and 4 weeks, not at 3 months. [2]$P < 0.05$, [3]$P = 0.055$. [4]Relative logMARΔ showed statistical significance at 1 month, not at 3 months.

Table 6: PPV versus T&I in present BAE series.

	PPV 29/83 (35%)	T&I 54/83 (65%)	*P* value
VA, pre-endophthalmitis	$n = 28$	$n = 53$	
Mean	20/55	20/50	0.3
Range	20/20-CF	20/20-LP	
VA, presentation	$n = 30$	$n = 54$	
Mean	LP	HM	0.02
Range	20/80-LP	20/40-NLP	
VA, 3 months	$n = 27$	$n = 39$	
Mean	3/200	20/390	
Range	20/25-NLP	20/25-LP	
logMARΔ	1.23	0.57	0.02

Table 7: PPV versus T&I: presentation VA of LP or worse in present BAE series.

	PPV 18/26 (69%)	T&I 8/26 (31%)	*P* value
VA, pre-endophthalmitis	$n = 18$	$n = 8$	
Mean	20/65	20/270	0.16
Range	20/20–1/200	20/25-HM	
VA, presentation	$n = 18$	$n = 8$	
Mean	LP	LP	0.35
Range	LP	LP-NLP	
VA, 3 months	$n = 17$	$n = 5$	
Mean	1/200	1/200	
Range	20/60-NLP	20/200-LP	
logMARΔ	1.71	1.18	0.46

months in eyes treated with IVD [8]. The present series was unique as it was the first to study BAE cases that had a higher rate of *Streptococcus* and Gram-negative cases.

BAE studies are limited by the relatively small number of BAE cases. Conclusions in BAE studies are found in the inherent limitations of retrospective nonrandomized data. The majority of cases in the present study received IVD which is similar to other BAE series [9–12]. Overall the two groups compared in this study had similar baseline demographic, clinical presentation, and initial culture data (Table 1).

A difference was found in the initial treatment of the two groups. The majority of cases in both groups received T&I as the initial treatment, however the percentage that received initial PPV was higher in the IVD group. The effect this difference had on the VA outcomes is unclear. A comparison of PPV and T&I cases in this series showed that PPV cases had significantly worse mean VA at presentation and 3 months (Table 6).

When presentation of VA was LP or worse, 3-month-logMARΔ was worse in the PPV group but not significantly (Table 7).

As VA outcomes with PPV were worse than T&I in this series, it is unlikely that improved VA outcomes in the IVD group were due to a higher percentage of initial treatment with PPV.

The repeat culture results in this series were similar to the Endophthalmitis Vitrectomy Study (EVS). In the EVS, 14 of 420 (3.3%) had positive repeat cultures [14]. In the present

series the overall repeat culture-positive rate was 3 of 83 (3.6%). Of note the EVS did not use IVD and the EVS had a lower rate of *Streptococcus* and Gram-negative organisms, yet the rate of persistent infection was similar in the EVS to the present study. The present study confirms the observation made by Shaarawy et al. that persistent infection can occur in bacterial endophthalmitis and appears to be more common with virulent organisms such as *Streptococcus* and *Enterococcus* [15]. Although persistent infection occurred in the present BAE series there was not a higher rate among the cases treated with IVD.

VA outcomes in the present series confirm the clinical observation by Irvine et al. that intraocular steroids appeared to hasten visual recovery [2]. At 1 month, 67% gained ≥3 lines in the IVD group compared to 25% in the group that did not receive IVD. The VA gains in the IVD group were more significant at 1 month than 3 months. Logistic regression did show that IVD was a predictive factor of ≥3 lines of improvement at both 1 and 3 months. VA gains may have been due to a decrease in intraocular inflammation, but a standardized manner of grading inflammation was not employed in the present series.

Limitations of the current study include the retrospective nature, small sample size in the control arm, and lack of a definitive treatment protocol. This study does demonstrate that IVD was associated with improved short-term VA outcomes and did not potentiate infection in BAE.

Disclosure

The authors in this paper have no financial or proprietary interests in products, methods, or materials published in this paper.

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Research Article

Clinico-Microbiological Profile and Treatment Outcome of Infectious Scleritis: Experience from a Tertiary Eye Care Center of India

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Medical and microbiology records of seventeen patients (17 eyes), diagnosed as scleritis of infectious origin were reviewed; to study clinical features, predisposing risk factors, microbiologic profile and treatment outcome of infectious scleritis. The mean patient age was 52.3 ± 19.75 years. Twelve patients (70.6%) had history of trauma/prior surgery. Isolated organisms included *Staphylococcus* species (spp) ($n = 5$), Fungus ($n = 4$), *Nocardia* spp ($n = 3$), two each of atypical *Mycobacterium* spp and *Streptococcus pneumoniae* and one *Pseudomonas aeruginosa*. Treatment included intensive topical antimicrobial in all eyes and systemic medication in 15 (88.2%) patients; surgical exploration was needed for 13 (76.5%) patients and scleral patch graft was done in four (23.5%) patients. Lesions resolved in all patients and none required evisceration. The presenting log MAR visual acuity of 1.77 ± 1.40 and improved to 0.99 ± 0.91. ($P \leq 0.039$) after treatment with a mean follow up of 22.57 ± 19.53 weeks. A microbiological confirmation, appropriate medical and/or surgical intervention has a good tectonic and visual outcome.

1. Introduction

Infectious scleritis presents as an ulcerated or nonulcerated, inflamed scleral nodule [1]. It accounts for 5–10% of all cases of scleritis [2–5]. But the presenting picture of infectious scleritis may not differ too much from immune-mediated scleritis [6–10]. Approximately 40–90% of immune mediated scleritis have an associated systemic vascular disease [3, 4, 6, 10]. While systemic treatment with corticosteroids or immunosuppressant benefits immune-mediated scleritis, it worsens the infectious scleritis. Hence it is imperative to differentiate between two conditions.

Many authors have reported infection by *Pseudomonas aeruginosa* and fungus as the most common causative organism [1, 11–13]. Pterygium surgery with beta radiation or application of mitomycin C has been identified as a common risk factor for infectious scleritis [11–13].

The clinical outcome is generally poor and most cases required evisceration in the many series [8, 9, 14]. Systemic and topical medication combined with early surgical intervention have improved the anatomical success, but not the visual outcome in two other series [1, 12].

Infectious scleritis is a rare entity; hence it is not suspected at the initial presentation resulting in delayed diagnosis and treatment. In this communication, we describe the predisposing factors, clinical features, etiology, and treatment outcome of infectious scleritis.

2. Material and Method

We retrospectively reviewed the medical and microbiological records of all patients with microbiologically proven infectious scleritis examined at Cornea Services of L V Prasad Eye Institute Bhubaneswar from November 2006 to August 2009. At presentation, all patients had received a detailed ophthalmic examination in the office. Patients with ulcerative lesions had received a scraping with a no. 15

surgical blade on a Bard Parker handle from the base and active edges of the ulcer under topical anesthesia in the office, and all nonulcerative nodular abscesses had received scleral scraping in the operating room under peribulbar anesthesia. In the later case, the base of the lesion was scrapped after dissecting the conjunctiva and deroofing of the nodular lesion. Materials collected from the lesions were smeared on glass slides and stained with Potassium hydroxide + Calcoflour white stain, Grams stain, Zeihl Neelsen stain using 20% H_2SO_4 or modified Zeihl Neelsen stain using 1% H_2SO_4. Acid fast staining was usually done if Grams stain smear was negative, but the lesions were strongly suspicious of microbial origin. The exudates from the lesion were cultured on blood agar, chocolate agar, Sabouraud's dextrose agar (SDA), Brain-heart infusion broth and non-nutrient agar with an *Escherichia coli* overlay. All media were incubated at 37°C except SDA; this was incubated at 27°C. Significant growth was defined as confluent growth on solid media, and/or there was growth of the same organism on more than one medium, and/or growth in one medium was accompanied by presence of similar organism in smears. All bacteria and fungi grown were identified as per standard protocol, and bacteria were tested for antibiotic susceptibility by Kirby-Bauer disc diffusion method.

The initial therapy either was based on results of smear or when smear was negative, an empirical treatment with topical antibiotic (mostly gatifloxacin 0.3% and amikacin 2.5%) along with systemic gatifloxacin ((400 mg) twice daily) was given. The treatment was modified, if needed, based on final culture and sensitivity report. Surgical debridement was done should the scleral ulceration area extend locally or progressed to form a subconjunctival abscess at another site away from the main lesion.

The collected retrospective data included patient demography (age, gender, occupation), disease history (onset, course, predisposing factors), clinical feature, type of organisms, antibiotic susceptibility, treatment given, and the outcome.

3. Results

We included 17 patients (17 eyes) of infectious scleritis between November 2006 and August 2009. The inclusion criteria were presentation with a scleral ulcer and/or abscess and an organism isolated microbiologically. This included 7 women and 10 men; the age ranged from 13 years to 75 years (mean 52.3 ± 19.75 years, median 55 years). (Table 1) The mean followup was 22.57 ± 19.53 weeks (range of 3–89 weeks).

The most common predisposing factor was an ocular surgery [$n = 9$; 52.9%. 95% (CI), 29.27–76.73]. The surgery included cataract in seven eyes, scleral buckle in one eye, and trabeculectomy in one eye. The interval from surgery to diagnosis of infectious scleritis ranged from one week to four years. Four patients had injury with organic material like wood and mud three weeks to seven months prior to presentation (mean 37 ± 33.04 days; median 21 days). Fifteen of 17 patients were using topical corticosteroids and two patients were using oral corticosteroids at the time of reporting to us. Only one patient was diabetic in this series.

The symptoms present in all patients were redness, pain, and watering in the affected eye. The presenting visual acuity varied from hand motions (HM) to a normal vision of 20/20. Twelve patients (70.54%) presented with a vision less than 20/40. Unifocal or multifocal scleral abscess was seen in six patients (35.39%) (Figures 1(a) and 1(b)). Characteristically, the abscesses presented as yellowish nodules under the intact conjunctival epithelium. Scleral ulceration and necrosis was seen in eight patients (47.05%) (Figures 1(c) and 1(d)). Necrosis around the incisional area was seen in all seven patients who had received cataract surgery earlier. Three eyes (17.34%) had both ulceration and abscess (Figure 2(a)).

The culture grew a variety of organisms. They included *Staphylococcus* species ($n = 5$; 29.41%), fungus ($n = 4$; 23.52%), *Nocardia* species ($n = 3$; 17.6%), *Streptococcus pneumoniae* ($n = 2$), atypical *Mycobacterium* ($n = 2$), and *Pseudomonas aeruginosa* ($n = 1$). One of the four Staphylococcus was methicillin resistant (no. 7). Of the four fungi isolated, two could not be identified, one belonged to Fusarium and Paecilomyces species each.

The results of antibiotic susceptibility testing for bacterial isolates (Kirby Bauer disc diffusion method) are given in Table 2. *Staphylococcal* and *streptococcal* cases were treated with fortified Cefazolin (50 mg/mL) and a flouroquinolone (ciprofloxacin 0.3%/gatifloxacin 0.3%) along with systemic fluoroquinolones (ciprofloxacin 500 mg/gatifloxacin 400 mg twice daily). Patient no. 7 was treated only with topical ciprofloxacin. Fungal scleritis was treated with topical natamycin 5% and systemically either itraconazole (100 mg) or ketoconazole (200 mg) two times daily. *Nocardia* and atypical *Mycobacterium* scleritis were treated primarily with topical fortified amikacin (25 mg/mL). Systemic trimethoprim (160 mg) sulphamethoxazole (800 mg) (TMX-SMZ) combination was used in patients of *Nocardia* scleritis only.

Thirteen of the 17 patients underwent wound debridement. During the surgical debridement, it was noticed that the area actually involved was generally much larger than initially seen under slit lamp. N-Butyl Cyanoacrylate glue was used in two patients as there was a limbal perforation of less than 1 mm × 1 mm in size. Five patients received scleral patch graft; as primary procedure in one (patient no. 4, Figure 1(c)), and the remaining four eyes, 2 days to one month after initiation of medical treatment. Seven eyes needed multiple surgical interventions.

Oral corticosteroid (1 mg/kg of body weight) was used in 5 eyes with bacterial scleritis two days after antibacterial treatment. In case no. 4, scleral patch graft was done as the primary procedure, later fungal filaments were identified from smear; so corticosteroids were started only after two weeks when recurrence was not noticed. Intraocular antibiotic was used in three patients suspected to have endophthalmitis. All of them presented scleral infection after cataract surgery.

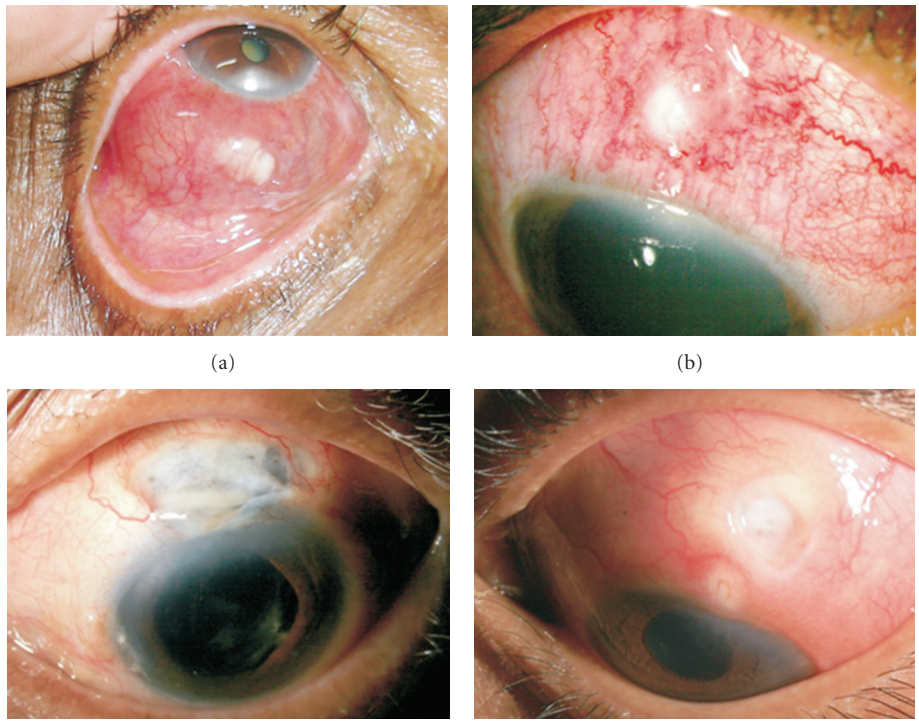
Resolution was defined as absence of symptoms, congestion, or active infiltrate. All the patients in this series responded to treatment; 13(76.47%) patients had only scarring with no or minimal uveal show; one patient

Table 1: Patient demographic, clinical, and microbiological feature, management, and outcome.

Sl. no.	Age	Sex	Eye	History	Duration of symptoms (in days)	Presenting visual acuity	Clinical signs	Organism	Treatment			Final visual acuity	Remarks	Outcome
									Topical	Systemic	Surgical			
1.	72	M	OS	Cataract surgery	45	CF 2 m	Necrosis, corneal infiltrate	*S. aureus*	F. cefazolin, ciprofloxacin	Ciprofloxacin	TA + BCl	20/70	Corneal scar	Resolved
2.	65	M	OD	Nil	60	20/40	Abscess	*Nocardia spp.*	F. amikacin, gatifloxacin	TMX-SMZ	Exploration -2	20/60p	Cataract	Resolved
3.	19	F	OS	Nil	150	20/25	Punched out ulcer	*Atypical mycobacterium*	F. amikacin, ciprofloxacin	TMX-SMZ, prednisolone	Nil	20/30	Corneal infiltrate, choroidal	Resolved
4.	72	M	OS	Cataract surgery	45	CF 2 m	Necrosis	*Fusarium spp.*	Natamycin, gatifloxacin, cyclosporine	Itraconazole	SPG	20/120	Graft vascularized	Resolved
5.	35	F	OD	Injury (with stick)	15	CF 2 m	Abscess, corneal infiltrate	Fungus	Natamycin, gatifloxacin	Itraconazole	Exploration, Intracameral ampho- B	20/160	Corneal scar and cataract	Resolved
6.	66	M	OD	Cataract surgery	9	HM	Scleral necrosis, exudate	*S. pneumoniae*	F. cefazolin, gatifloxacin, betamethasone	Lizolidine, predinisolone	SPG + IOAB	20/60	Graft vascularized	Resolved
7.	49	M	OD	Nil	120	20/20	Ulcer, abscess, thinning	*Staphylococcus spp.*	Ciprofloxacin, prednisolone	Prednisolone	Nil	20/20	Nil	Resolved
8.	50	M	OS	Cataract surgery	7	HM	Necrosis	*S. aureus*	F. cefazolin, ciprofloxacin	Gatifloxacin	SPG + Vitreous biopsy + IOAB	20/100	Graft vascularized, corneal scar	Resolved
9.	74	F	OS	Injury (with mud)	15	HM	Abscess	*P. aeruginosa*	F. amikacin, Ciprofloxacin, Prednisolone	Ciprofloxacin, prednisolone	Exploration	20/200	Cataract, choroidal	Resolved

Table 1: Continued.

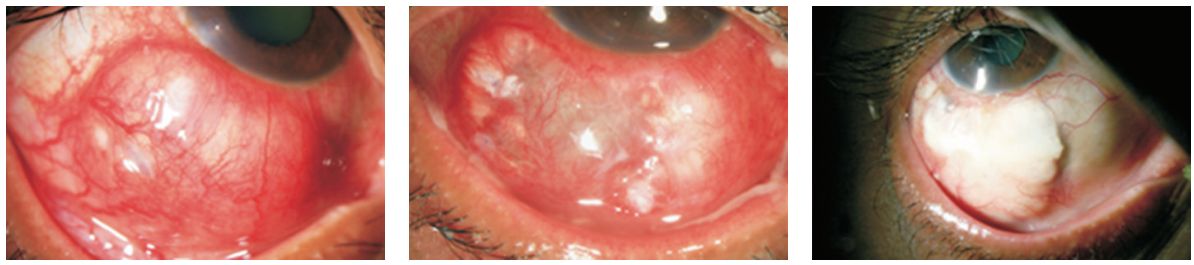
Sl. no.	Age	Sex	Eye	History	Duration of symptoms (in days)	Presenting visual acuity	Clinical signs	Organism	Treatment			Final visual acuity	Remarks	Outcome
									Topical	Systemic	Surgical			
10.	50	F	OD	Injury (with twig)	75	20/30	Chemosis, abscess	*Nocardia spp.*	F. amikacin, ciprofloxacin	Ciprofloxacin	Exploration SPG + AMG	20/20	Graft vascularized	Resolved
11.	13	M	OD	Trabeculectomy	30	CF 1 m	Abscess	*S. aureus*	F. cefazolin, gatifloxacin	Cefadroxil	Nil	CF 1 m	Glaucoma, optic atrophy	Resolved
12.	21	M	OS	Thyroid Ophthalmoplegia	Not available	20/20	Abscess	*S. pneumoniae*	F. gentamicin, chloramphenicol	Prednisolone	Nil	20/30	Nil	Resolved
13.	53	M	OS	Retinal detachment surgery (buckle)	60	CF 1 m	Ulcer, abscess	*Atypical mycobacterium*	F. amikacin, gatifloxacin	Gatifloxacin	BB Removal	HM	Elevated Granuloma	Resolved
14.	55	F	OS	Nil	60	20/200	Thinning, ulcer, abscess	*Nocardia spp.*	F. amikacin, ciprofloxacin	Ciprofloxacin, TMX-SMZ	Exploration -3	20/50	Nil	Resolved
15.	75	F	OD	Cataract surgery	60	HM	Necrosis	Fungus	Natamycin	Itraconazole	TA + BCL	20/50	Corneal scar	Resolved
16.	65	F	OS	Cataract surgery	7	20/70	Necrosis, corneal infiltrate	*S. aureus*	F. cefazolin, gatifloxacin, prednisolone	Ciprofloxacin	Wound repair + IOAB	20/40	Membrane over IOL	Resolved
17.	55	M	OS	Cataract surgery	30	20/160	Necrosis	*Paecilomyces spp.*	Natamycin	Ketoconazole	SPG	PL+	Pupillary membrane	Resolved



(a) (b)

(c) (d)

Figure 1: Slit lamp picture depicting different clinical presentation. (a) Case no. 2: multiple scleral abscess. (b) Case no.12: single scleral abscess. (c) Case no. 4: necrotic ulcer (post cataract surgery). (d) Case no. 3: two punched out ulcers.



(a) (b) (c)

Figure 2: Slit lamp picture of Case no. 10. (a) At Presentation showing multiple abscesses and ulcer. (b) Three weeks after initiation of treatment (surgical exploration and medical management), there is a uveal show. (c) Final outcome showing vascularized scleral graft (four months after scleral patch graft).

required scleral patch graft (Patient no. 10 Figures 2(a), 2(b), and 2(c)) one month after initiation of treatment. In the patients where scleral patch graft was done, success was defined as no evidence of graft or surrounding infiltrate and vascularization of the graft. All five grafts were healthy at the last visit.

The mean presenting and post treatment logMAR visual acuity was 1.77 ± 1.40 and 0.99 ± 0.91. ($P \leq 0.039$) (Figure 3). The vision improved by greater than 2 lines in 8 of 12 patients who presented with a visual acuity of <20/40 patients.

The primary cause of decreased vision after resolution of infection was cataract and corneal scar. Total choroidal detachment developed in two patients and was treated with tapering oral corticosteroids. One of the two patients had atypical *Mycobacteria* infection. This 19-year-old lady also developed a corneal abscess after seven months of treatment. It resolved with topical fortified amikacin.

4. Discussion

Scleritis may represent a diagnostic challenge and is often associated with life-threatening systemic disease (in this series, only one patient had diabetes mellitus though) and vision-threatening ocular complications [15].

Scleral infection from *Pseudomonas*, *Staphylococcus*, or Herpes zoster virus can cause necrotizing scleritis, which is clinically identical to systemic autoimmune disease [6].

Table 2: Results of antibiotic susceptibility testing for bacterial isolates (Kirby Bauer disc diffusion method).

Sl. no	Patient no. (from Table 1)	Name of the bacteria	Antibiotics						
			Chlo	Cefa	Vanco	Cipro	Gati	Oflo	Amik
1	1	*S. aureus*	S	ND	ND	S	S	ND	ND
2	2	*Nocardia* sp.	S	S	ND	S	S	ND	S
3	3	Atypical mycobacteria	S	S	S	S	S	ND	S
4	6	*S. pneumoniae*	S	S	S	S	S	S	ND
5	7	*Staphylococcus* sp.	S	S	S	R	S	I	ND
6	8	*S. aureus*	S	S	S	I	I	I	ND
7	9	*P. aeruginosa*	R	ND	ND	S	S	S	S
8	10	*Nocardia* sp.	R	R	R	S	S	S	S
9	11	*S. aureus*	R	S	S	R	I	I	ND
10	12	*S. pneumoniae*	S	S	S	S	S	S	ND
11	13	Atypical mycobacteria	R	R	R	R	R	R	S
12	14	*Nocardia* sp.	S	R	S	R	S	R	S
13	16	*S. aureus*	S	S	S	I	S	I	ND

Chlo: Chloramphenicol, Cefa: Cefazolin, Vanco: Vancomycin, Cipro: Ciprofloxacin, Gati: Gatifloxacin, Oflox: Ofloxacin, Amik: Amikacin, S: Sensitive, I: Intermediate, R: Resistant, ND: Not done.

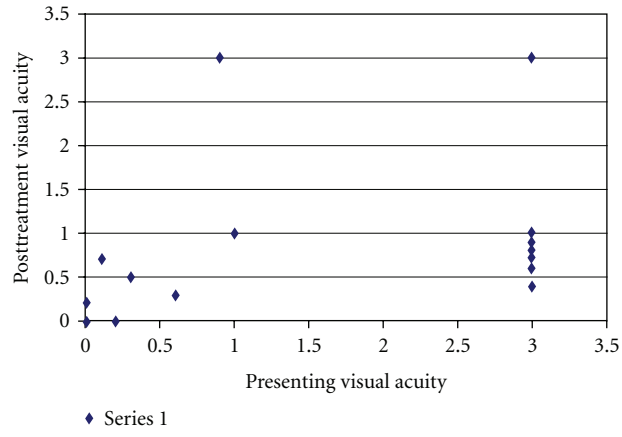


Figure 3: Comparision of the presenting visual acuity with posttreatment logMAR visual acuity of all patients.

Unusual organisms like *Nocardia, Acanthamoeba,* atypical *Mycobacteria*, *Mycobacterium tuberculosis*, and *Listeria monocytogenes* are known to cause scleritis [1, 16–18]. Often the diagnosis of an associated infection or systemic condition dictates therapy. Systemic vasculitis typically requires systemic corticosteroid or immunosuppressive drugs [2, 3]. An early and definitive diagnosis helps in treatment of the condition and has better outcome.

Patients with a history of prior ocular surgery or trauma and presenting with a scleral abscess or ulceration and necrosis should arouse the suspicion of infectious origin. The surgeries include pterygium, cataract, scleral buckling, and strabismus surgery [10–12, 18]. In our series, cataract surgery was the predisposing factor in seven eyes and trauma with organic material was present in four eyes.

Corticosteroids, given before infection control, in an infectious scleritis worsen the condition by inhibiting release of lysosomal enzyme. In this cohort, 15 of 17 patients were on topical corticosteroids at presentation to us. It is unknown whether this aggravated the infection or decreased the immunity for secondary infection to occur.

Bacterial infectious scleritis is more common than other infectious scleritis. *P. aeruginosa* has been the often reported infecting organism [10, 12, 13, 15]. It was reported in over 50% eyes from Taiwan following pterygium surgeries [12, 13]. In our cohort, however, *Staphylococcus* infection (24.41%) and cataract were the most common surgery (41.17%). In our series, only one of 17 patients had *P. aeruginosa* infection.

One of our coauthors has reported fungal infectious scleritis in over one of third patients and high incidence of *Nocardia* infection from Hyderabad (south central India) [1]. In this series, we detected fungus in close to a quarter of patients and *Nocardia* in close to 20 percent. High incidence of fungus in both series can be attributed to the hot and humid climate and the enormous amount of fungal spore prevalent in the environment [19]. Two of the three patients with *Nocardia* scleritis could not recollect any history of injury although one of them was an agriculturist. All three patients with *Nocardia* scleritis required multiple explorations of the abscess, and one patient (no. 10) (Figures 2(a), 2(b), and 2(c)) required a scleral patch graft after resolution of the active infection.

In addition to topical medications, systemic antibiotic or antifungal was required for 15 patients. Systemic corticosteroids were used in patients with bacterial infection ($n = 5$), in eyes that developed choroidal detachment ($n = 2$), and in the patient who developed exophthalmous (no. 12).

Pyogenic infections of the sclera are often difficult to eradicate because of poor antimicrobial penetration into the avascular necrotic sclera; combination of surgery such as abscess exploration and systemic and topical antimicrobial therapy yields superior results [1, 13, 20, 21]. Surgical

debridement not only facilitates penetration of antibiotic but also debulks the infected scleral tissue.

We do agree with Lin et al. who suggested mandatory surgical exploration of the abscess that does not respond to initial medication to increase the penetration of antibiotic [13]. Raber et al. reported a "tunnel lesion" on histopathological finding in cases of scleral ulcer [22]. Our experience is similar to Lin et al. who also described the tunnel lesion and recommended the need of careful exploration to avoid residual infection [13]. Two (no. 2, 14) of our 7 patients in this series required multiple surgical intervention and both of them had "tunnel lesion".

All 17 patients in this series achieved anatomical success. Fourteen of 17 patients (82.35%) regained useful vision (defined as vision ≥20/200). The causes of poor vision in the remaining three eyes were glaucomatous optic atrophy (no. 11), chronic retinal detachment (no. 13), and fibrotic membrane in the pupillary area (no. 17). In an earlier series by one of our coauthors, one-third of patients had regained useful vision, but three of 21 eyes were eviscerated and one eye became phthisical [1]. Eleven of the 18 eyes in Hsiao et al. series retained useful vision, three had poor vision and four had to be eviscerated [12]. None of our patients in this series required evisceration.

Associated uveitis is not uncommon. (66% in Su et al. series) These inflammations lead to formation of pupillary membrane, cataract, and endophthalmitis [10]. Most patients in our series had low-grade inflammation in the anterior chamber at presentation; severe intraocular inflammation occurred in five patients subsequently. Cataract was the most common sequel in our series ($n = 3$). Four patients who had extension to cornea resolved with corneal scar.

Our series has all weaknesses inherent to all retrospective studies. Our institution is a tertiary care referral center, so there is a potential for bias towards more unusual or difficult-to-control diseases. Nonetheless, we have demonstrated that a variety of organism can cause scleritis. An early clinical suspicion of infectious origin, identification of the infecting organism, knowledge of antibiotic susceptibility, and institution of appropriate medical and/or surgical therapy could have good tectonic and visual outcome.

Conflict of interests

The authors declare no conflict of interests.

Acknowledgment

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