A Preliminary Study to Treat Severe Endophthalmitis via a Foldable Capsular Vitreous Body with Sustained Levofloxacin Release in Rabbits

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Purpose. We investigated whether the foldable capsular vitreous body (FCVB) could release levofloxacin sustainably in vitro and inhibit endophthalmitis in rabbit models.

Methods. Approximately 1.0 mL levofloxacin (625 μg/mL) was injected into the capsule of nine FCVBs. The levofloxacin release value was determined in the modified Franz diffusion cells over time. In the in vivo study, all right eyes of 45 rabbits were infected with *Staphylococcus epidermidis* and were divided randomly into three groups at 24 hours after infection: FCVB plus levofloxacin ($n = 15$), silicone oil plus subconjunctival levofloxacin ($n = 15$), and an untreated group ($n = 15$) during a 30-day observation time. Levofloxacin concentrations in the aqueous humor were detected, and therapeutic efficacy was evaluated with clinical evaluation, bacterial counts, cytokine profiles, and histopathology.

Results. The FCVB released levofloxacin ranging from 9 to 13.5 ng/mL in vitro and from 42 to 1.6 ng/mL in the aqueous humor during 30 days. In the FCVB and silicone-treated groups, clinical inflammation almost was abolished; no bacteria were detected in the aqueous humor; TNF-α, IL-1β, and IFN-γ expression decreased; and relatively normal corneal and retinal architecture were kept after the 30-day treatment.

Conclusions. The FCVB could provide us with dual functions, combining a levofloxacin drug delivery system and a vitreous substitute, to treat endophthalmitis in rabbit eyes. (*Invest Ophthalmol Vis Sci.* 2013;54:804–812) DOI:10.1167/iovs.12-9695

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Current drug delivery systems (DDS), such as liposomes, biodegradable microspheres and nanospheres, high molecular weight polymers, and mechanical pumps, have only one function, that is sustainable release of drug to maintain therapeutic concentrations at specific locations in the body with minimal side effects.[1] Moreover, the carriers of current DDS only match one drug via chemical combination. For many retinal disorders, including endophthalmitis,[2] proliferative vitreoretinopathy,[3] and proliferative diabetic retinopathy,[4] the challenge is to develop combinations of DDS and vitreous substitutes. To date, strategies for combining these two functions are lacking.

Endophthalmitis is the most serious complication of ocular surgery and it also may arise after penetrating ocular trauma, or from systemic or periocular infection.[5] The clinical presentation of endophthalmitis is variable, from a mild and therapeutically responsive inflammation to complete vision loss or loss of the eye itself, despite prompt and often aggressive therapeutic, and surgical intervention. The recommended management for bacterial endophthalmitis includes injecting antibiotics directly into the vitreous. The most commonly used therapeutic combinations for intravitreal injections have included vancomycin (1.0 mg) and amikacin (0.4 mg) or ceftazidime (2.2 mg). Many clinicians prefer to substitute ceftazidime for amikacin because toxicity to retinal cells has been reported following amikacin use.[6] However, ceftazidime is incompatible physically with vancomycin, and the two antibiotics must be injected from separate syringes to avoid precipitation of the drugs.[7] Levofloxacin has activity against Gram-positive and Gram-negative bacteria.[8] Previous studies have established the safety and efficacy of levofloxacin as a treatment for conjunctivitis and keratitis,[9,10] and for intravitreal levofloxacin in *Staphylococcus epidermidis* endophthalmitis.[11,12] A single injection of intravitreal antimicrobial agents may be insufficient to cure some cases of endophthalmitis, and a second injection should be considered.[13] Considering this, developing DDS for treating endophthalmitis is increasingly important not only to enhance drug efficacy, but also to reduce side effects.

For severe endophthalmitis, vitrectomy combined with silicone oil tamponade might be beneficial in the treatment strategy of severe endophthalmitis.[14–16] Vitrectomy is an appealing adjunct to management, which often is used to debride and remove the nidus of infection, as well as provide much more space for antibiotics, especially in those cases involving intraocular foreign bodies.[17] Meanwhile, retinal detachment is one of the most serious complications of endophthalmitis and occurs with a 21% incidence[18] after vitrectomy procedures due to inadequate support to the retina. In response to these problems, artificial vitreous substitutes should be implanted to refill the vitreous cavity, improve anatomic stabilization, and reduce the risk of retinal detachment. Silicone oil has been used in the repair of retinal

detachment and has activity against the endophthalmitis-causing agents.[19,20]

In our previous studies, we proposed a new strategy to fabricate a vitreous substitute with a novel foldable capsular vitreous body (FCVB).[21–26] The FCVB, made with modified liquid silicone rubber, consists of a thin, vitreous-like capsule finely mimicked by computer, and a tube-valve system. After the FCVB capsule is folded and implanted in the vitreous cavity, a balanced salt solution (BSS) and/or therapeutic drugs then can be injected into the capsule, and the capsule inflated to support the retina.[21] As a vitreous substitute, the FCVB can be used for retinal detachments by providing a solid arc, especially in the inferior retina as well as multiple retinal detachments that silicone oil was unable to support adequately due to its low density. Meanwhile, it could avoid the disadvantages of silicone oil, including glaucoma, keratopathy, and emulsification,[27–30] and induce very little refractive shift.[22,23]

Interestingly, numerous 300 nm tiny apertures were observed in the FCVB capsule, and we uncovered its potential use as a DDS for the ophthalmic drug dexamethasone sodium phosphate in the rabbit eye.[24] Moreover, we determined that the human FCVB could release levofloxacin in vitro,[25] as well as the rabbit FCVB could release levofloxacin in vitro and vivo.[26] Here, we tested further whether the FCVB could serve as a vitreous substitute and DDS in the rabbit endophthalmitis model.

Materials and Methods

In Vitro Drug Sustained-Release Studies

Nine FCVBs were injected separately with 1.0 mL levofloxacin solution at concentrations of 625 μg/mL, and then the capsules were immersed in cups of modified Franz diffusion cells. The 200 μL of liquid in the cup were aspirated for measurement at 10, 20, 40, 60, 120, 180, 240, 300, and 360 minutes.[24] The levofloxacin content in the liquid was then detected by sensitive high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS; Thermo Finnigan LLC, San Jose, CA).

Endophthalmitis Induction

S. epidermidis (ATCC12228) was grown overnight at 37°C on trypticase soy agar. The bacteria were harvested via centrifugation, washed two times, and suspended in sterile 0.9% NaCl. The suspension was diluted further to 2.0×10^6 CFU/0.1 mL for intravitreal injections.

A total of 45 New Zealand albino rabbits weighing 2 to 3.5 kg was maintained in accordance with institutional guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Anesthesia was induced with an intramuscular injection of a 50/50 mixture of ketamine hydrochloride (50 mg/kg) and xylazine (10 mg/kg). Pupillary dilation was achieved with 1 drop of tropicamide 1%. The fundus was examined in all rabbits before injection, to rule out any baseline retinal disease. All intravitreal injections were performed approximately 3 mm posterior to the limbus with a 30-gauge needle. Aqueous humor (0.1 mL) was removed by paracentesis before the intravitreal injection to prevent an increase in intraocular pressure.[31]

Treatment Protocol

At 24 hours after infection, the rabbits were divided randomly into three treatment groups as follows: FCVB plus levofloxacin ($n = 15$), silicone oil plus levofloxacin injected subconjunctivally ($n = 15$), and the untreated group ($n = 15$). Before all surgical procedures, ocular inflammation was graded based on an ophthalmic grading system from Pleyer et al.[32] as described in Table 1. For the FCVB group, pars plana lensectomy (PPL) and vitrectomy (PPV) were performed on the right eye of each rabbit using a vitrectomy machine (ACCURUS; Alcon Laboratories, Inc., Fort Worth, TX), and then the FCVB was implanted in the right eye of 12 rabbits.[24] Approximately 1.0 mL levofloxacin (625 μg/mL) then was injected into the capsule through a silicone tube-valve system, and thus, the capsule was inflated to support the retina. For the silicone group, the same PPL and PPV were performed with the same vitrectomy machine. After the vitrectomy, the silicone oil was injected into the vitreous cavity; subsequently, daily subconjunctival injections of 1.0 mL levofloxacin (625 μg/mL) were administrated. In contrast, 12 animals in the untreated group at 24 hours after receiving intravitreally inoculated bacteria were not managed at all.

Table 1. Quantitative Clinical Grading Score (Basis for Assigning Points in Quantitative Clinical Grading)

Cornea
0 = Clear
1 = Focal edema
2 = Diffuse edema
3 = Moderate opacity (iris visible)
4 = Severe opacity (bare iris detail)
5 = Opaque (no iris view)
Anterior chamber
0 = Clear
1 = Trace protein flare
2 = Mild protein flare
3 = Moderate protein flare, mild fibrin/hypopyon
4 = Severe strands, hemorrhage
5 = Fibrin/hypopyon (no iris detail), hemorrhage
Iris
0 = Normal
1 = Mild hyperemia
2 = Moderate hyperemia
3 = Severe hyperemia, synechia
4 = Marked hyperemia, synechia, irregular pupil
5 = Neovascular
Vitreous
0 = Clear
1 = Trace haze
2 = Areas of vitreous haze, some fundus details visible, good red reflex
3 = View of vessel outlines only
4 = Moderate vitreous haze, no fundus details visible, partial red reflex
5 = No red reflex

In Vivo Drug Sustained-Release Studies

Aqueous humor (approximately 0.1 mL) was removed from the anterior chamber of three rabbits on days 1, 3, 5, 10, 20, and 30 in the two treated groups. The aqueous samples were frozen immediately and used to determine the levofloxacin concentration by LC-MS/MS as described above. The only difference was that 50 μL of aqueous humor samples also were transferred into 2.0 mL Eppendorf tubes with prepared standard working solutions at the desired concentrations.

Clinical Examination

Examinations of the eyes with a slit-lamp and an indirect ophthalmoscope when necessary were made in all groups on days 0, 5, 10, 20, and 30 after treatment. A clinical score was given to each eye as described earlier. All scores then were averaged to determine the mean score. Since 3 animals were sacrificed at days 0 and 5 for the cytokines in the ciliary body and retina (see below), clinical grading was done in 12 eyes on day 0, in 9 eyes on day 5, and in 6 eyes on days 10, 20, and 30.

Analysis of Cytokine mRNA by Real-Time PCR

In each group, 3 eyes on days 0, 5, and 30 after treatment were obtained. Total RNA was extracted from the ciliary body and retina with Trizol (Takara, Dalian, China) and reverse transcription of purified

Table 2. Primers for PCR Amplification of Specific Rabbit Cytokines

Cytokine	Accession No. (Gen-Bank)	Forward Primer	Reverse Primer
TNF-α	M12845	TTGTCCGTGAGCTTCATGCC	AAGCCTCTAGCCCACGTAGTAG
IL-1β	D21835	GCCACTGTGGTAAGCCATCATC	AGGCAAGAGGCACAACAGATCG
IFN-γ	AB010386	TCAGCCTCACTCTTCTGAAGC	TCTGGTTAGTGTG TCCTGGCAG
β-actin	AF309819	CATGTACGTGGCCATCCAG	TCTTCATGAGGTAGTCGGTCAGGTC

RNA was performed. The quantification of gene transcripts was measured by real-time PCR, with the LightCycler (Roche, Basel, Switzerland) and SYBR RT-PCR kit (Takara). We obtained data from three independent experiments. The mRNA levels of TNF-α, IL-1β, and IFN-γ were compared after they had been normalized relative to those of β-actin. Rabbit genes and primer pairs used are listed in Table 2.

Microbiologic Analysis

On days 0, 5, 10, 20, and 30, samples of the aqueous humor (approximately 1.0 mL) from 3 right eyes in each group were 10-fold serially diluted, plated for quantification on blood agar and incubated at 37°C for 24 hours. After the incubation period, the surface colonies were counted and identified as *S. epidermidis*. Colonies were counted using the agar spread plate technique. The microbiologists were masked to the experimental status of the eyes from which the aspirates were obtained.[33]

In addition, on day 5, three eyes of three rabbits in each group were obtained. Ciliary and retina tissues were ground, and then dilutions of each sample were done in 500 μL physiologic saline for all specimens. Then, 100 μL aliquots of each dilution were placed in blood agar petri dishes. Plates were incubated at 37°C for 48 hours. CFU then were counted and recorded.

Histopathology

Three animals from the FCVB group and three from the silicone oil group on day 30, and three animals from the untreated group on day 10 (rabbits in the untreated group were observed until 10 days for complications) were euthanized and then enucleated after the eye examination. The globes were fixed in 10% buffered formaldehyde for 96 hours. All eyes were sectioned through the optic nerve, and any gross abnormalities were recorded. Specimens were stained with hematoxylin and eosin (H&E), and examined with a microscope.

Statistical Analysis

Multiple group comparison was performed using ANOVA. A nonparametric Mann-Whitney *U* test was performed for the clinical score, and an unpaired, 2-tailed Student's *t*-test was used to determine the statistical significance for the cytokine. A P value ≤ 0.05 was considered statistically significant.

Results

Levofloxacin Release Characterization In Vitro and In Vivo Studies

Rabbit FCVB can release levofloxacin mechanically by osmotic pressure without early burst effects in vitro. The results showed that the released levofloxacin was 9 ng/mL at the 10-minute time point and then stabilized as 12 ng/mL over the following observation period (Fig. 1A). The relationships between concentration (Q) and time ($t^{1/2}$) were described with the Higuchi equation: $Q = 0.1475t^{1/2} + 10.1090$ ($r = 0.667$). Our study revealed that, in vivo, levofloxacin was released sustainably from the FCVB in the aqueous humor. We found that the values of the FCVB with 1 mL levofloxacin and 625 μg/mL in the endophthalmitis model were 42, 31, 12.5, 5.6, 2.5, and 1.6 ng/mL on days 1, 3, 5, 10, 20, and 30, respectively, and that release concentration decreased gradually over time. Levofloxacin still was detectable at 1.6 ng/mL on day 30 (Fig. 1B). In the silicone oil group, the levofloxacin concentrations in aqueous humor were 71.7, 340, and 371 ng/mL, respectively, on days 1, 3, and 5. This administration method can reach the inhibitory concentration in aqueous humor on 3 day (Fig. 1C).

Analysis of Therapeutic Effect by Clinical Observation

On day 0, namely at 24 hours postinjection, clinical inflammation degree in the three groups had no statistical difference between the FCVB- and silicone oil–treated groups and the untreated group ($P > 0.05$, Fig. 2A). On day 5, most rabbits in the FCVB group showed moderate exudation in the anterior chamber and opaque optical media. The inflammatory

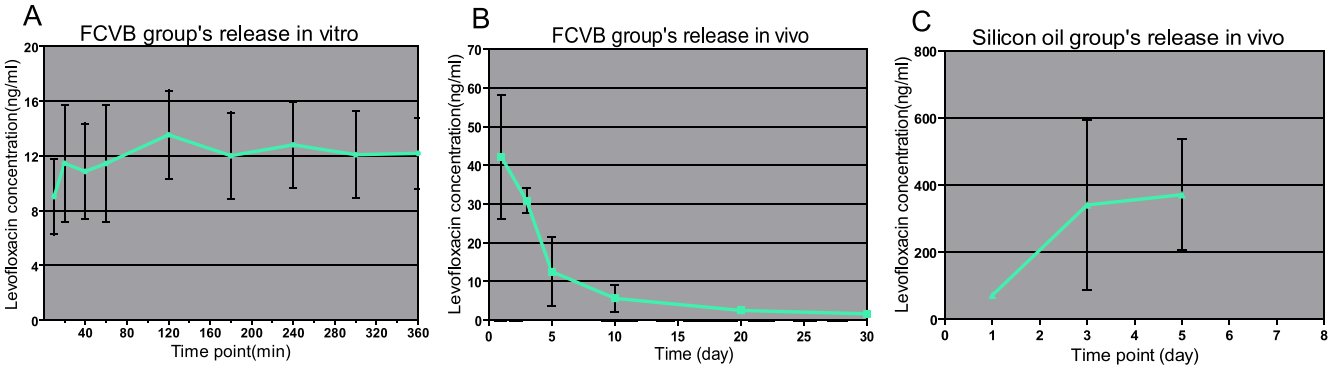

Figure 1. Levofloxacin release characterization in vitro and in vivo. (**A**) The levofloxacin released from the FCVB in vitro was 9 ng/mL at the 10-minute time point and then stabilized as 12 ng/mL over the following observation period. (**B**) FCVB continuously released levofloxacin in vivo. The levofloxacin in the aqueous humor was 42, 31, 12.5, 5.6, 2.5, and 0.6 ng/mL, respectively, on days 1, 3, 5, 10, 20, and 30. (**C**) The aqueous concentration of levofloxacin in the silicone oil group. The levofloxacin in the aqueous humor was 71.7, 340, and 371 ng/mL, respectively, on days 1, 3, and 5. *Error bars* represent the SEM.

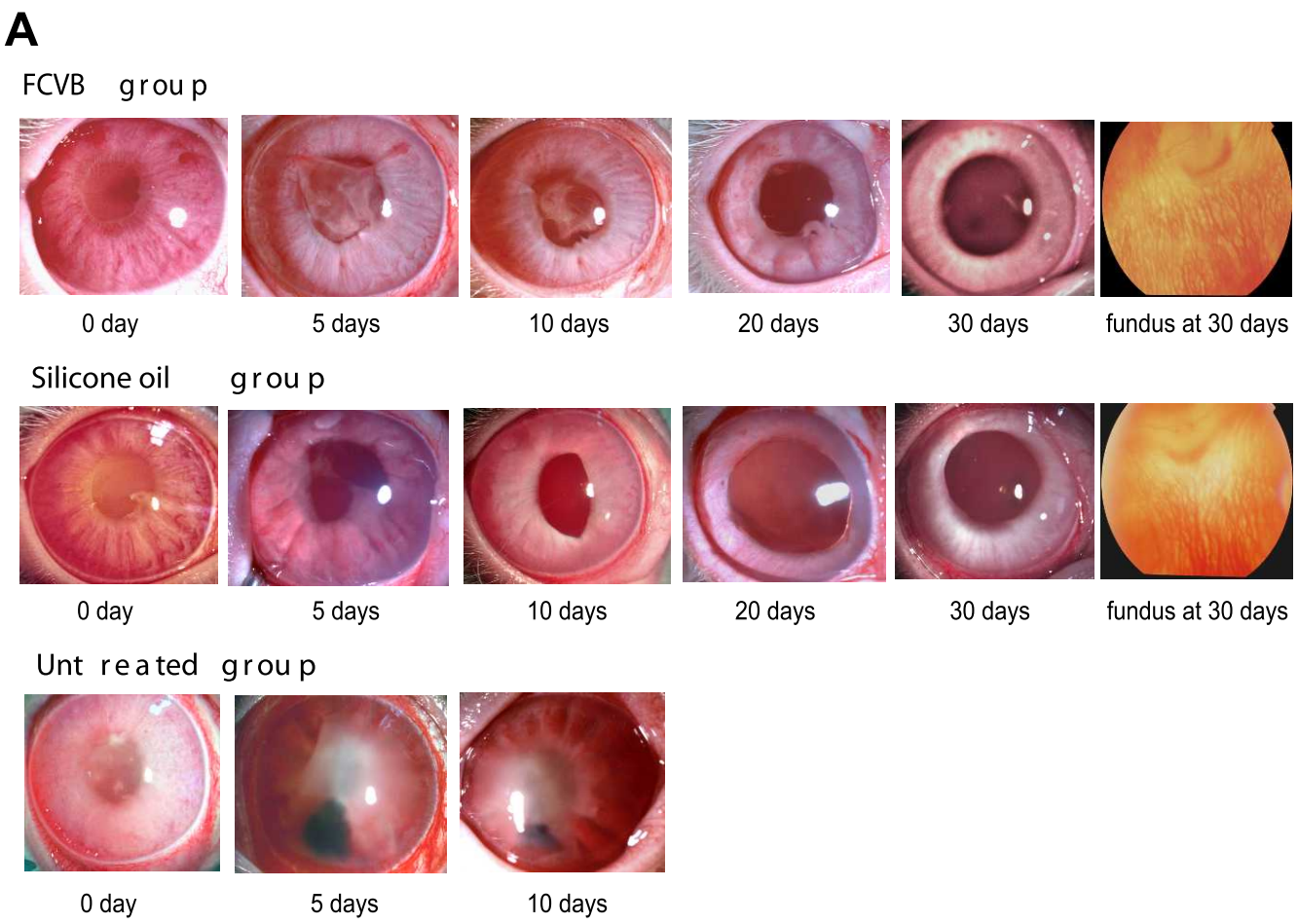

Figure 2. Analysis of therapeutic effect by clinical observation. (**A**) Photographs of the rabbit endophthalmitis eyes in the two treatment groups on days 0, 5 10, 20, and 30 post-treatment, and the untreated group on days 0, 5, and 10. The five photos in the two treatment groups on the *left* show the anterior eye, the one on the *right* shows the fundus of eye. In the untreated group, an intraocular photograph could not be obtained due to the opaque anterior segment. No signs of endophthalmitis were observed in the FCVB- and silicone oil–treated eyes at the end time point. (**B**) Clinical inflammatory scores in the three groups are presented as mean clinical scores ($n = 5$). The *error bars* represent SEM. At the beginning stage, the inflammatory scores in the FCVB group were higher than in the silicone oil group, but declined with time and reached similar scores as the silicone oil group.

scores in this group and in the untreated group had no significant difference ($P > 0.05$). In relation to the silicone oil–treated rabbits, there were lower inflammatory scores ($P = 0.005$ and 0.008 with the untreated and FCVB groups, respectively). After day 10, exudation in the FCVB group decreased gradually with time, and the inflammatory scores demonstrated a significant decline. There was no difference between the FCVB group and silicone oil group ($P > 0.05$). However, the inflammatory scores in the untreated group did not show improvement ($P = 0.001$ and 0.000 with the FCVB and silicone oil groups, respectively). Rabbits in the untreated group were observed for 10 days for complications (one rabbit suffered periorbital cellulitis). On day 20, the two experimental groups showed a continued decrease in inflammatory scores. Finally, by day 30 post-treatment, the clinical inflammation was almost abolished in the two treated groups. Mean scores in the two treated groups were almost the same ($P > 0.05$, Fig. 2B). The score chart of rabbits in each group was showed in Table 3.

B-scan ultrasonography showed some mild opacity echoes in the vitreous cavity and a smoothly increased epiretinal echogenicity in all FCBV-treated eyes, which appeared to be the posterior wall of the FCVB supporting the retina (see Supplementary Material and Supplementary Fig. S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.12-9695/-/DCSupplemental). There was interference of the silicone oil in the silicone oil–treated eyes during the 30 days.

Table 3. Chart of Clinical Examination Scores in Each Experimental Group at the Different Time Points

	Pretreatment	Posttreatment Score by Days			
Group No.	**Score (0 day)**	**5**	**10**	**20**	**30**
FCVB group					
1	12	13	11	9	3
2	13	12	9	5	2
3	12	11	6	3	1
4	15	14	10	4	1
5	16	15	12	8	2
6	16	16	14	8	
7	13	12			
8	12	12			
9	15	15			
10	13	14			
11	11	7			
12	16	14			
13	14				
14	13				
15	15				
Silicone oil group					
1	14	9	6	3	1
2	11	8	5	2	1
3	12	9	7	3	1
4	13	5	4	4	3
5	13	9	7	3	2
6	14	9	6	4	1
7	13	6			
8	14	6			
9	15	6			
10	16	7			
11	13	9			
12	12	10			
13	15				
14	14				
15	13				
Untreated group					
1	10	12	15		
2	13	14	14		
3	14	15	15		
4	13	14	18		
5	14	15	15		
6	15	15	15		
7	13	14			
8	15	15			
9	12	14			
10	13	13			
11	12	15			
12	12	13			
13	13				
14	16				
15	11				

Analysis of Therapeutic Effect by Cytokine Determination

In the ciliary body, TNF-α, IL-1β, and IFN-γ expression decreased on days 5 and 30 compared to day 0 in the silicone oil group. TNF-α and IFN-γ were above day 0 levels on day 5 in the FCVB group ($P < 0.05$), but below day 0 on day 30. The factor IL-1β always was below day 0 on days 5 and 30 (Fig. 3A).

In the retina, TNF-α, IL-1β, and IFN-γ levels on days 5, 30 were lower than those on day 0 in the silicone oil–treated groups ($P< 0.05$). In the FCVB group, IFN-γ levels were above day 0 levels on day 5 ($P < 0.05$), but below day 0 on day 30. The other two factors always were below day 0 on days 5 and 30 (Fig. 3B).

Bacterial Load and Histopathologic Analysis

Bacterial counts were not observed in aqueous samples from both eyes of the FCVB and silicone oil groups in which pars plana lensectomy (PPL) and vitrectomy (PPV) had been performed. The aqueous chamber and vitreous cavity were unblocked in both groups, so the results could suggest that there were no bacteria in the entire eye. In the untreated group, no bacteria were observed in the aqueous humor. Animals in this group did not have their lenses removed and there were no bacteria in the aqueous humor; this result is similar to that of other reports.[33]

In addition, the tissue culture of the ciliary body and retina on day 5 showed nearly no bacteria survival in the FCVB- and silicone oil–treated groups, but still has approximately 4.0×10^4 CFU organisms in the untreated eyes.

Histopathology was shown in Figure 4. On day 30, no obvious abnormality was observed in the corneas in the FCVB- and silicone oil–treated groups. However, in the untreated group, the corneal stromal layer showed obvious edema. In the FCVB-treated eyes, there were some inflammatory cells and a few infiltrated interstitial cells in the ciliary body, disorganized structure, and marked vessel dilation and cellular infiltrate in the retina. In the silicone oil group, a few ciliary epithelial cells and few inflammatory cells appeared in the ciliary body, and there was less disruption in the retina. In contrast, dissolved necrosis occurred in the ciliary body and retina on day 10 in the untreated group due to endophthalmitis. In addition, a great number of inflammatory cells were observed around the optic nerve, in the posterior segment, and near the ciliary body.

Discussion

In our study, we demonstrated for the first time to our knowledge that FCVB can have dual functions to release

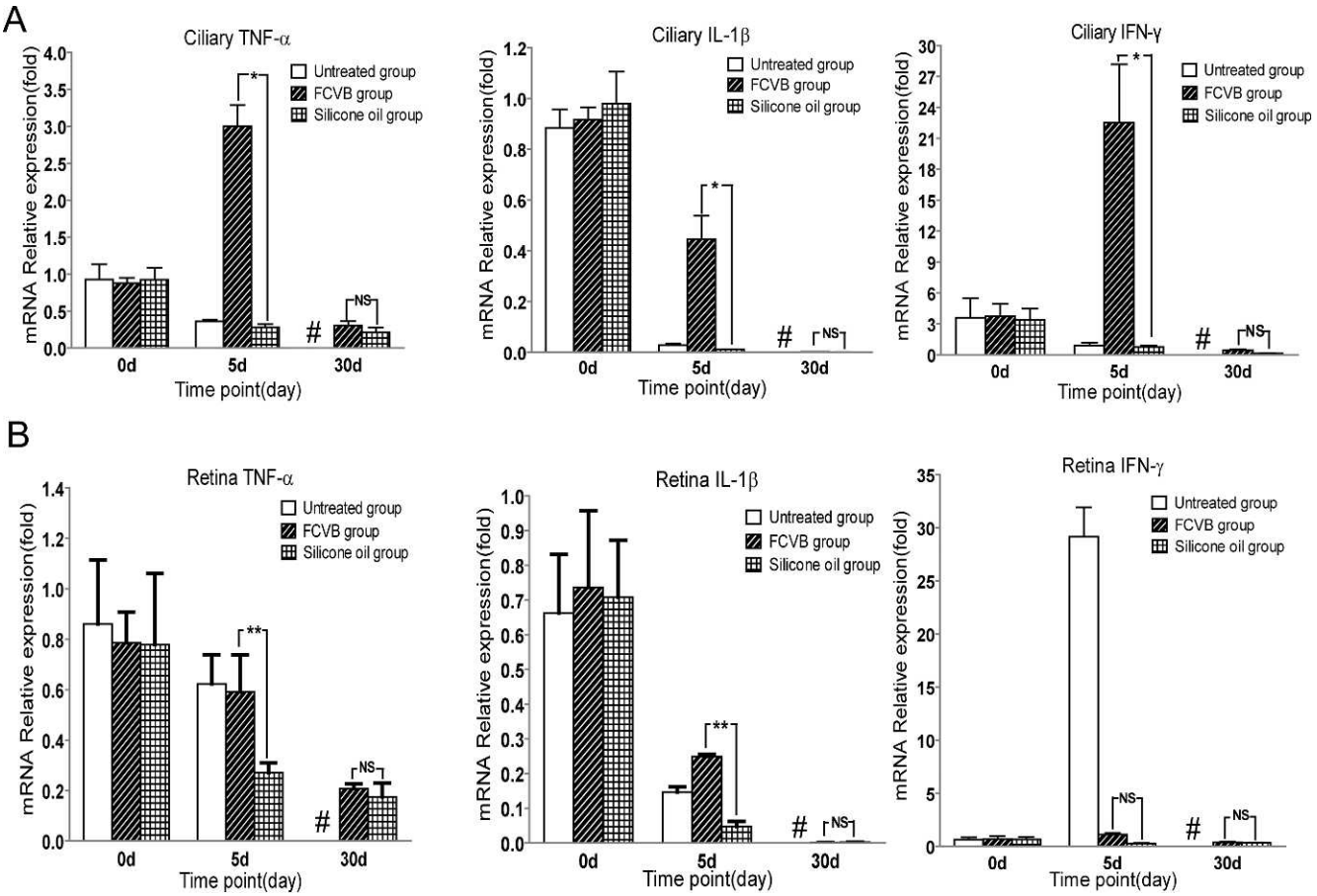

Figure 3. Analysis of therapeutic effect by inflammatory cytokine determination. Comparison of the levels of rabbit inflammatory cytokines in ciliary body (**A**) and in retina (**B**) as determined with SYBR RT-PCR. Histograms representing the fold-changes ($2^{-\Delta\Delta CT}$) in the quantitation of cytokines in the FCVB group and silicone oil group compared to the untreated group. Samples collected on days 0, 5, and 30 after treatment. Values represent the mean SEM for $n = 3$ per group. #, indicates that rabbits in the untreated group were not detected after 10 days due to severe complications. $^{**}P < 0.001$; $^{*}P < 0.05$; NS, not significant.

levofloxacin mechanically and sustainably to treat endophthalmitis, and act as vitreous substitutes in a rabbit model.

Previous studies have shown that injecting levofloxacin intravitreally was effective in killing bacteria and suppressing intraocular inflammation in a variety of infectious conditions.[11,12] However, levofloxacin is eliminated rapidly from the eye after direct intravitreal injection, with the minimum inhibitory concentration for 90% of isolates (MIC90) of levofloxacin for major pathogens of endophthalmitis lasting less than 48 hours (Ohkubo S, et al. *IOVS* 2004;45:ARVO E-Abstract 3951). The chronic and recurrent nature of endogenous endophthalmitis usually necessitates sustained therapeutic tissue levels of antibiotics. Because numerous 300 nm tiny apertures exist in the FCVB capsule,[24] levofloxacin, with a molecular mass of 415.85 daltons (Da), may penetrate the apertures in capsule easily by passive diffusion. Kazi et al. established the level of toxicity of intravitreal levofloxacin; the doses of 625 μg or less of levofloxacin in 0.1 mL injected into the midvitreous of rabbits had no signs of retinal or vitreal toxicity.[34] Therefore, we chose 625 μg/mL levofloxacin in our study.

In our study, we showed that levofloxacin was released from the implant, and penetrated the vitreous cavity and aqueous humor. Our in vitro studies revealed that the amount of apertures restricts the total flow rate and limits the rapid loss of the levofloxacin, and then the drug's release is sustained. In our previous study, the in vitro release of levofloxacin more than doubles from a human FCVB with concentrations of 500 μg/ml.[25] The reason probably is that the sizes and apertures in human FCVB are 3 times those in rabbits. The in vivo release rate was relatively faster than the in vitro release rate. This may be as a result of the decreased osmotic pressure around the capsule of FCVB by the released levofloxacin taken away by blood, or metabolized quickly in vivo. Since the osmotic pressures in the capsule of FCVB are consistent between in the vitro and in vivo studies, the only difference is the osmotic pressure around the capsule.

The aqueous humor concentration represents the drug concentration distributed into the anterior chamber following release from the FCVB in the vitreous cavity. In our previous study, we reported that the release values of the FCVB with 500 μg/mL levofloxacin in normal eyes were 132, 50, 39, 11, and 15 ng/mL on days 1, 7, 14, 28, and 56, respectively.[26] In our study, we found that the values with 625 μg/mL levofloxacin in endophthalmitis eyes were 42, 31, 12.5, 5.6, 2.5, and 0.6 ng/mL on days 1, 3, 5, 10, 20, and 30, respectively. Although similar release tendency appeared, release values in normal eyes were bigger than those in endophthalmitis ones probably due to inflammation.

In the present study, we showed that the time courses of cytokine TNF-α, IL-1β, and IFN-γ expression levels were consistent with the clinical inflammation presentation. This

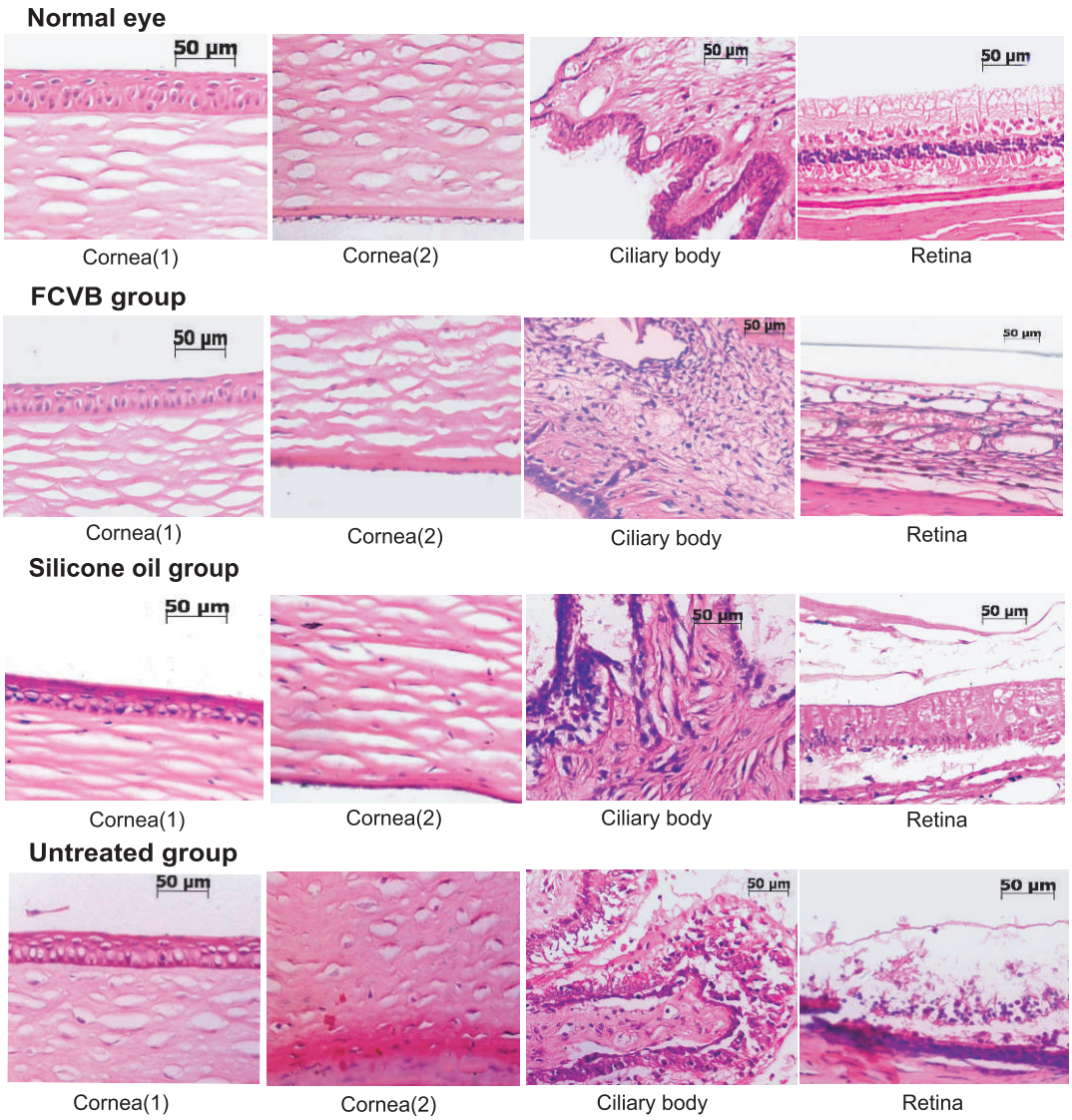

Figure 4. Histopathologic analysis of representative eyes. Photomicrograph of rabbit eyes on day 30 after treatment in the two treatment groups and on day 10 in the untreated group. Cornea (*1*) shows the corneal epithelium; and cornea (*2*) shows the corneal endothelium. No obvious abnormality observed in the corneas in the FCVB- and silicone oil–treated groups. However, in the untreated group, the corneal stromal layer showed obvious edema. In the FCBV-treated eyes, there were some inflammatory cells and a few infiltrated interstitial cells in the ciliary body, disrupted structure, and marked vessel dilation and cellular infiltration in the retinas. In the silicone oil group, a few ciliary epithelial cells and few inflammatory cells appeared in the ciliary body, and less disruption in the retina. In contrast, dissolved necrosis occurred in the ciliary body and retina on day 10 in the untreated group due to endophthalmitis. In addition, a great deal of inflammatory cells were observed around the optic nerve, in the posterior segment, and near the ciliary body. H&E, magnification: ×200.

result also confirmed previous work.[35] At the beginning stage, the clinical inflammation and level of cytokine expression in the FCVB group were significantly higher than those in the silicone oil group, maybe because the amount of levofloxacin released was not enough to reduce the inflammatory process in the early stages of treatment. However, the difference between these two groups disappeared over the 30 days examined.

We showed that the clinical inflammation on day 10 in the untreated group was higher than in the FCVB- and silicone oil–treated groups. Additionally, the eyes in the untreated group had an opaque vitreous on ophthalmoscopy, a severe inflammatory cell infiltration in the vitreous and retina, and a disintegrated retinal architecture. In contrast, the eyes of the two treated groups had clear vitreous, a relatively normal retinal appearance by the ophthalmoscopic examination, and a normal level of cytokines by real-time PCR, though the retinal architecture had a moderate degree of disorganization in the FCVB group eyes. Since there is no mechanical injury when the FCVB is implanted in a noninflamed eye,[29] the causes of the retinal edema and disorganization are more likely due to the effects of the inflammatory process.

Recently, studies have demonstrated that the major problem inherent in the use of silicone oil as vitreous tamponade is emulsification,[36] and emulsification can generate oil droplets that may cause secondary glaucoma, keratopathy, and subjective disturbances, even after the silicone oil is removed.[37] Although silicone oil is a relatively effective vitreous substitute, it cannot support the inferior retina due to its low density.[38,39] Our previous studies demonstrated that the FCVB has no obvious complications induced commonly with traditional silicone oil,[27] and can be used for retinal detachments that

were not reattached easily with silicone oil, such as inferior retina and multiple retinal detachments.[28–30] An exploratory clinical trial conducted at Zhongshan Ophthalmic Center in China suggested that an FCVB was a flexible, effective, and safe vitreous substitute over a 3-month implantation period in 11 human eyes[28] and over a 12-month implantation in 3 human eyes.[30] Currently, multiple center clinical trials are in progress to ascertain FCVB efficacy and safety as a novel vitreous substitute in nine hospitals in China. In our study, the FCVB sustainably released levofloxacin and had an effect similar to silicone oil combined with levofloxacin in suppressing microbial activity, though the silicone oil/levofloxacin group reduced inflammation in an earlier stage compared to the FCVB group.

Colthurst et al. considered that the best solution appears to be to develop a material used in the posterior segment of the eye that is an effective tamponade agent and that can provide intravitreal drug delivery.[38] In our study, the FCVB worked as a combination of DDS and vitreous substitute, permitting the levofloxacin in the capsule to permeate outside stably, and effectively treating endophthalmitis. Therefore, FCVB is a potential new approach for the future, combining a DDS and a vitreous substitute.

We determined that the MIC of *S. epidermidis* (ATCC12228) isolate is 200 ng/mL for levofloxacin by broth dilution method, which is consistent with the report of Yamada.[40] Lower drug concentrations were released from the FCVB over longer duration. In fact, fewer bacteria remained following vitrectomy. The efficacy of bacterial inhibition is cumulative because the drug is released sustainably from FCVB. So, the inhibitory concentration of residual bacteria in the vitreous cavity might be lower than that of one-time administration dosage for full bacteria. The exact amounts of drug need further research. A higher than 625 μg/mL (1 mL) drug load may achieve similar efficacy as bolus injections of the drug solution. However, potential retinal toxicity should be considered over 625 μg/mL in the eye once the FCVB broke because significant decreases in electroretinography were recorded in the previous study injected with 1250 and 2500 μg levofloxacin.[34]

In our study, vitrectomy removed most pathogens before the FCVB was implanted or silicone oil was injected. On this basis, the FCVB with sustained levofloxacin release could accomplish a similar bacterial inhibition role as silicone oil.[19,20,41] Moreover, the FCVB, can be used for other drugs, including dexamethasone sodium phosphate[24] and protein kinase C alpha.[42] Therefore, FCVB results also may have implications in the treatment of important retinal diseases that need drug and vitreous substitutes, such as age-related macular degeneration, proliferative vitreoretinopathy, and proliferative diabetic retinopathy. This work, along with our other reports regarding the FCVB,[21–30,42] give an example of how to make the product from bench to bedside, including idea, craft, animal model, producing standard, technical reports from government, clinical trails (exploratory, multiple centers), and marketing. Based on this study, we will apply for a new clinical trial to test the combined function of FCVB.

In summary, our study suggests that the FCVB could release levofloxacin mechanically and sustainably in vitro and in vivo by osmotic pressure. It also could inhibit endophthalmitis effectively in rabbits, and so provides us with a novel research and therapeutic strategy for the management of endophthalmitis combining a DDS and a vitreous substitute.

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