Experimental Intracameral Injection of Vancomycin Microparticles in Rabbits

Laurent Kodjikian,1,2 Jérémy Couprie,1 Walid Hachicha,3 Quadiri Timour,4 Mojgan Devouassoux,5 Nicolas Builles,6 Daniel Hartmann,2 and Hatem Fessi3

PURPOSE. To evaluate the in vivo toxicity and efficacy of previously developed poly(lactide-co-glycolide)-vancomycin–based microparticules (V-MPLs) for eventual use for endophthalmitis prophylaxis during cataract surgery.

METHODS. The intraocular vancomycin concentration profile was evaluated after V-MPL injection into the anterior chamber of rabbit eyes. The toxicology of V-MPLs versus MPLs alone was tested by corneal cellular counting and retinal histology. The prophylactic efficacy of the V-MPLs was evaluated by bacterial counts after introducing contaminated intraocular lenses (IOLs) together with the V-MPLs into one anterior chamber of phakic rabbit eyes or without V-MPLs in control rabbit eyes.

RESULTS. Intraocular V-MPLs produced effective vancomycin concentrations over at least 6 hours. Corneal counts revealed no significant increase in dead cells. Retinal toxicity manifested as inflammation 3 hours after injection, reaching its maximum between 12 hours and 24 hours, decreasing by 48 hours, and completely disappearing at 72 hours. Inflammation was similar between V-MPLs and MPLs. Untreated eyes implanted with highly infected IOLs showed severe, reproducible endophthalmitis. No sign of infection was observed with infected IOLs and concomitant V-MPL treatment, supported by bacterial counts showing a significant decrease in colony-forming Staphylococcus epidermidis units in the anterior chamber and on the implant surfaces within 6 hours.

CONCLUSIONS. The present study demonstrated the release and toxicologic properties of the authors’ newly developed V-MPLs in vivo. In addition, the rabbit model shows that V-MPLs are effective in reducing the risk of experimental endophthalmitis. (Invest Ophthalmol Vis Sci. 2010;51:4125–4132) DOI:10.1167/ iovs.09-4694

Endophthalmitis is a rare and severe bacterial infection of the eye that can result in serious eye damage and possible vision loss. Prophylactic antiseptics and antibiotics are frequently used before surgery to reduce bacterial populations on the ocular surface.1–8 Despite these measures, the frequency of positive bacterial cultures from anterior chamber aspirates ranges from 0% to an impressive 46%, and postoperative endophthalmitis still occurs at a prevalence of 0.07% to 0.38%.5–9 The only randomized controlled trial proving the efficacy of a prophylactic antibiotic was the European Society of Cataract Refractive Surgery (ESCRS) multicenter study,10 which reported a fivefold lower incidence of endophthalmitis in patients given an intracameral ceftoxime bolus injection at the end of surgery. However, the bacteria most often responsible for endophthalmitis display a non-negligible rate of ceftoxime resistance.11,12

Vancomycin is a bactericidal antibiotic that inhibits bacterial cell wall synthesis with 100% efficacy against Gram-positive organisms that cause endophthalmitis.11,13 However, vancomycin added to the irrigation solution during cataract surgery prevented endophthalmitis.14 Indeed, it is now well established that intraocular vancomycin injected during cataract surgery fails to maintain effective antibacterial activity for an adequate period (at least 11 hours).15 Hence, other formulations are needed to sustain intracameral vancomycin concentrations sufficiently for the antibiotic to demonstrate its efficacy.

Controlled drug delivery with microparticles (MPLs) containing vancomycin were developed for topical use,16 but the antibiotic was released for 2 hours only. In previous work, we developed vancomycin (V)-MPLs especially for intracameral use by controlling the particle size and release profile.17 We subsequently tested these in vitro and demonstrated efficacy against Staphylococcus epidermidis.18

In the present study, we focused on the toxicity of V-MPLs in rabbits and evaluated their efficacy in preventing experimental endophthalmitis. To our knowledge, no previously published study has attempted to induce prophylaxis against endophthalmitis intracameral with a V-MPL suspension.

MATERIALS AND METHODS

Vancomycin, V-MPLs, and MPLs Alone

Vancomycin chloride (vancomycin) was purchased from Merck Généralités (Lyon, France). Sterile biocompatible vancomycin and vancomycin-free microparticles (MPLs) were especially designed for this study.17 The MPLs contained exactly the same components as the V-MPLs except for the antibiotic.

Bacterial Strain

A clinical isolate of S. epidermidis (N890074) was supplied by the microbiology department of Edouard Herriot Hospital (Staphylococci National Reference Centre, Lyon, France). The strain was isolated from the infected cerebrospinal fluid of a hydrocephalic child who had a ventriculo-peritoneal shunt.
The bacterial species were identified as follows: (1) by colony and microscopy morphology; (2) lack of coagulase activity in rabbit plasma (BioMérieux France, Craponne, France); (3) lack of a clumping factor (Staphyloplus; BioMérieux); and (4) according to a staph gallery (ID32, BioMérieux). Its Ica gene locus was identified by using polymerase chain reaction (PCR) amplification. The gene is known to encode the production of S. epidermidis polysaccharide antigens that mediate adhesion to biomaterials (PS/A) and between bacteria (PIA). The isolate was sensitive to vancomycin with a minimum inhibitory concentration (MIC) of 2 μg/mL and minimum bactericidal concentration (MBC) of 8 μg/mL. Bacterial concentrations for assays were adjusted to 10^8 CFU/mL. Bacterial concentrations for assays were adjusted to 10^8 CFU/mL to produce a sufficient count to induce endophthalmitis in animals. The IOLs were washed three times in a phosphate-buffered saline solution (PBS buffer, pH 7.8) to eliminate nonadhering bacteria. Finally, one IOL was implanted into the anterior chamber of the phakic right eye of a rabbit. The second IOL provided the control CFU level of adherent bacteria.

**Intraocular Lenses**

Forty-eight sterile hydrophobic acrylic IOLs with identical optical and overall diameters (5.5 and 12.5 mm) and refractive power (20 D) were provided by Alcon SA (Sainte-Claire Deville, France).

Each implanted IOL had a matched control. Both IOLs (including haptics) were incubated with a bacterial suspension for 2 hours at 37°C in a blood collection tube (Vacutainer; BD Biosciences, Le Pont-De-Clair, France). Suspensions were adjusted spectrophotometrically to 10^8 CFU/mL to produce a sufficient count to induce endophthalmitis in animals. Minimum inocula of 10^7 CFU/IOL was used to avoid interference with possible MPL-induced inflammation. The IOLs were washed with pentabarlothial 88 mg/kg IV (Dolethal; Vetoequinol, Lure, France) before aqueous humor aspiration and/or IOL explantation.

**Animal Care**

Male New Zealand White rabbits purchased from CEGAV (Saint-Mars-d’Egrenne, France) were kept in the vivarium for 1 week before the experiment began and weighed 2.25 kg at the start of the study. The experimental protocol was approved by the Claude Bernard Lyon 1 University Ethics Committee and by the National School of Veterinary Ethics Committee. It also adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Before intracameral interventions, the animals were routinely anesthetized with ketamine 400 mg/kg (Imalgene; Merial, Lyon, France) and xylazine 32 mg/kg (Rompun; Bayer, Puteaux, France) injected into the rear flank muscle, followed by topical proparacaine 0.5% anesthesia to each cornea. During long experiments they also received buprenorphine 0.05 mg/kg IM every 12 hours to reduce pain and distress. Ketoprofen was not used, to avoid interference with possible MPL-induced inflammation. The rabbits were euthanatized with pentobarbital 88 mg/kg IV (Dolethal; Vetoequinol, Lure, France) before aqueous humor aspiration and/or IOL explantation.

**Bacterial Counting**

Aqueous humor bacterial counts were performed by spreading 0.05 mL of aspirate over a nutritive agar plate (Tryptcise-Soja: BioMérieux). For IOL bacterial counts, the lenses were soaked in a PBS buffer, bound bacteria were dispersed by sonication at 47 kHz for 5 minutes (Branson; Branson Ultrasonic Corp., Danbury, CT), and the resulting suspension was vortexed, diluted, and spread over a nutritive agar plate. The process removes all adherent bacteria without affecting their viability. Bacterial counts were performed after 24 hours of incubation at 37°C. For aqueous humor, these were expressed as CFU/mliliter. For IOLs, however, because the surface area depended on diameter, haptic shape, and dioptic power, the areas were as given by the manufacturer. The results are expressed as log_{10} CFU/50 mm^2.

We obtained accurate comparison of the results by binding the same number of bacterial colonies to each IOL and achieving the same control value over successive implantations. Because it was impossible to satisfy both conditions simultaneously, we eliminated variation by using the ratio IOL CFU/control CFU applied to adherent bacterial colony counts at the different implantation times and to counts before implantation. Results of the calculations are expressed as log_{10} CFU/50 mm^2, indicating the in vivo evolution of adherent bacterial colonies.

**Surgery**

Intracameral injections into phakic right eyes of rabbits were made with a 30-gauge syringe, with the rabbits under general anesthesia, after an equivalent volume of aqueous humor volume was aspirated. The previously infected IOLs were then introduced into the anterior chamber under aseptic conditions. Sterile patches and povidone iodine 5% solution (Betadine; NAPP Laboratories, Cambridge, UK) were consistently applied to the eye surface. No cataract surgery was performed.

**Experiment 1: Vancomycin Dosage Feasibility**

Injections of 0.05 mL vancomycin solution (1 mg/mL) were made into the anterior chamber of rabbits right eyes. Three hours later, the rabbits were euthanatized, aqueous humor was aspirated, and vancomycin concentrations were determined by an HPLC dosage procedure, as described elsewhere. The procedure was repeated six times, with three HPLC determinations performed on each sample.

**Experiment 2: Vancomycin Intraocular Half-Life**

Injections of 0.05 mL vancomycin solution 0.05 mL vancomycin solution (80 µg/mL) 0.05 mL were made into the anterior chamber of rabbits’ right eyes. At 0, 0.5, 1, 1.5, and 4-hour intervals (six rabbits/interval), intraocular aqueous humor vancomycin concentrations were determined by the HPLC procedure.

**Experiment 3: Vancomycin Release, Clinical Efficacy, and Toxicity**

Two groups of rabbits received injections of 0.05 mL of V-MPL (1 mg/mL) suspension, or 0.05 mL of MPL (1 mg/mL) suspension into their right eyes. Subsequently, at 0.5, 1, 3, 6, 12, 24, 48, 72, and 168 hours, injected eyes (six rabbits/interval/group) were clinically graded by a board-certified ophthalmologist (blinded to treatments) using a slit lamp and indirect ophthalmoscope with pupil dilation (2.5% phenylephrine and 0.5% tropicamide). Slit lamp examination was used to rate the following: (1) blepharitis (i.e., eyelid margins with injection, suppurative, and hair loss); (2) conjunctivitis/scleral injection (i.e., conjunctiva and sclera with injection, hyperemia, and chemosis); (3) limbal injection (i.e., the degree of peripheral redness near the cornea); (4) iritis (i.e., color changes, change ratios, and light reactivity of the iris); (5) corneal clarity (i.e., the opacity resulting from infection); (6) hypopyon (i.e., the degree of inflammatory cells in the anterior chamber); (7) fibrin production (i.e., the amount of fibrin in the anterior chamber); and (8) anterior chamber cells (i.e., the number of cells and flare seen with the slit lamp light beam at 2 mm). Indirect ophthalmoscope with pupil dilation was used to rate inflammation of the vitreous and any retinal changes, as follows: (9) vitreous grade (i.e., ability to visualize the retina clearly); and (10) the retina with clear vitreous (i.e., the presence of abnormalities within the retina itself). All clinical manifestations were rated on a scale of increasing severity: 0, 0.25, 0.5, 1, 2, and 3, with a maximum score of 30.

After clinical ratings were completed, aqueous humor was extracted, vancomycin concentrations were determined by the same HPLC dosage procedure as in experiment 2, and intraocular vancomycin concentrations were calculated as changes over time.

The eyes were then carefully dissected from their orbits, and the corneas were cut under a laminated flux hood and placed in cornea prep medium (Eurobio, Les Ulis, France). Residual eye tissue was discarded.
immerged in formaldehyde for histology of primarily the iris, ciliary body, and retina.

The determination of the density and viability of the corneal endothelial cells was performed at an eye bank, with the same procedure as that used for human corneas and in accordance with the recommendations of the European Eye Bank Association (EEBA, 2006). The eye bank was not aware of the time after injection, the age of the rabbits, or any other experimental data. Corneal endothelial cell density was determined by optical microscopy using a 1-mm², 10×10-square graticule for direct counting (Leica Biomed microscope; Reichert, Vienna Austria, with CCD camera; Sony, Tokyo, Japan) after staining with trypan blue (0.4% sterile trypan blue; Edouard Herriot Hospital, Lyon, France) for 1 minute, and intercellular space dilation for 4 minutes with 0.9% wt/vol pyrogenic sterile NaCl (Aguettant, Lyon, France). The count was performed on two 1-mm² zones: one located in the central cornea and the other 2 mm away from the central point (within an 8-mm diameter). Results are expressed as the average cell count in the two zones (cells/mm²).

Apoptotic or dead cells retained trypan blue staining in their nuclei, whereas live cells eliminated the stain by active transport systems. Dead cells were counted in each of the 1-mm² areas.

Toxicity in the remaining eye tissues (excluding the cornea) was established by histology, conducted according to the following procedures. Each globe was formalin fixed, paraffin embedded, and sectioned horizontally through the optic nerve. A representative HES (hematoxylin, eosin, saffron) 3-μm section was then analyzed (treatment blinded) by light microscopy. Inflammation intensity was scored as: − (none), + (slight), ++ (mild), and +++ (severe).

**Experiment 4: MPL Prophylaxis Capability**

It was hypothesized that infected IOLs would induce experimental endophthalmitis in rabbits. Infected IOLs were implanted into the anterior chamber of the phakic right eyes of rabbits under general anesthesia and in aseptic conditions, as described previously. The procedure was repeated six times in two rabbit groups which were clinically examined daily over 7 days. One group received infected IOLs only. The other group received infected IOLs together with V-MPLs. At 6-, 24-, 48-, 72-, and 168-hour intervals (six rabbits/interval) the animals were euthanatized. The right eyes were washed with povidone iodine 5% solution and rinsed with sterile balanced salt solution (BSS; Alcon SA) before the IOLs were removed with sterile forceps through corneal incisions. Bacterial counts were performed on two 1-mm² zones: one located in the anterior chamber (rabbit eye) and the other 2 mm away from the central point (within an 8-mm diameter). Results are expressed as the average cell count in the two zones (cells/mm²). Apoptotic or dead cells retained trypan blue staining in their nuclei, whereas live cells eliminated the stain by active transport systems. Dead cells were counted in each of the 1-mm² areas.

**RESULTS**

**Experiment 1: Vancomycin Dosage Feasibility**

All HPLC dosage determinations showed clearly visible aqueous humor vancomycin peaks at our previously validated retention time (Fig. 1), with good resolution. In addition, the chromatographs showed a new peak relative to our standard vancomycin in vitro solution, revealing aqueous humor constituents absorbing at our detection wavelength of 280 nm. The latter peak in the chromatograph did not interfere with the vancomycin peak. We were justified, therefore, in using the HPLC dosage method in experiment 2.

**Experiment 2: Vancomycin Intraocular Half-Life**

The intracameral vancomycin half-life (rabbit eye anterior chamber) was determined after approximately 28 minutes (Fig. 2).

**Experiment 3: Vancomycin Release, Clinical Efficacy, and Toxicity**

**Vancomycin Release.** Table 1 shows that vancomycin released from the V-MPLs remained above the minimum inhibitory concentration for more than 6 hours (12 half-lives).

**Clinical Efficacy.** With V-MPLs or MPLs alone (Table 2) clinical assessments revealed no posterior segment abnormalities. Indeed, vitreous and retinal clinical scores were excellent in all experiments, as were corneal clarity results.

It was difficult to rate flare in the anterior chamber as the injected MPLs disturbed flare detection in all cases. However, after minute examination, we concluded that no flare occurred in any experiment.

![Figure 2](https://via.placeholder.com/150)

**Figure 2.** Intraocular vancomycin concentration after a single injection into a rabbit’s anterior chamber. Vancomycin solution (0.05 mL of 80 μg/mL) was injected into the anterior chamber. At 0-, 0.5-, 1-, 1.5-, and 4-hour intervals intraocular vancomycin concentrations were measured by HPLC.
Conjunctivitis and limbal injection were the most frequently encountered disorders. Both appeared within the first hour after injection of V-MPLs or MPLs and persisted beyond 72 hours, peaking between 24 and 48 hours. Blepharitis, fibrin production, and iritis began between 6 and 12 hours, reached maximum intensity between 24 and 48 hours, and disappeared after 72 hours. Hypopyon also occurred, reaching maximum intensity at 24 hours and spontaneously disappearing completely by 72 hours. All abnormalities were observed equally with MPLs and did not differ significantly between treatments, indicating that inflammation was probably caused by the MPL component and not by the vancomycin molecules.

**Toxicity.** *Corneal Endothelium Density and Viability.* At 0.5 hour after intraocular V-MLP injection, a slight decrease in viable corneal cells was noted, relative to the control, that was not statistically significant ($P = 0.98$; Wilcoxon test). Dead cells were seen, but were not visible 1 hour after injection. In addition, the number of viable cells was constant when standard deviations were considered. No statistically significant difference was found for any cell count (Table 3, Fig. 3). Also, no dead cells were seen after injecting MPLs, the number of viable cells remained constant, and no statistically significant differences were observed (data not shown).

**Iris and Retina Histology.** Results after V-MLP and MPL injections were strictly identical with respect to abnormalities and when they were observed (Fig. 4).

Between 0.5 and 3 hours after V-MLP or MPL injections, histologic studies showed no abnormalities and no inflammatory cells. Subsequently, eyes presented signs of acute anterior uveitis with inflammatory neutrophil infiltration of the iris stroma and ciliary body. The stroma was edematous and contained neutrophils without necrosis. No foreign body reaction or granulomatous inflammatory element were seen. There was no neovascular membrane or proliferation of pigment epithelium in the iris. Eyes with more severe inflammation (score, $+++$) showed a fibrinous exudate with neutrophil leukocytes at the iris surface. There was, however, no tissue destruction or disorganization, and iris structure remained intact, despite the intense acute inflammation. The posterior segment showed no histologic lesion. Inflammation intensity was scored + at 3 hours, $++$ at 6 hours, and $+++$ at 12 and 24 hours and then lessened after 48 hours (score $+$) and disappeared at 72 hours.

**Experiment 4: MPL Prophylaxis Capability**

Implantation of infected IOLs ($\sim 10^5$ CFU/IOL) without antibiotics into the anterior chamber of a phakic rabbit eye caused severe reproducible endophthalmitis from day 1 that persisted for 1 week (i.e., a few hours after injection, the appearance of conjunctival and limbal injection, hypopyon, and fibrin in the anterior chamber were assumed to indicate endophthalmitis). Also, intracameral bacterial and IOL colony counts were present until 72 hours and then declined, presumably because of specific nutriment deprivation and possibly the hosts’ de-

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**Table 1.** Intraocular Vancomycin Concentration after Single Intracameral Injections of Microparticles

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<th>Time (h)</th>
<th>Intracameral Vancomycin Concentration (µg/ml)</th>
<th>SD</th>
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<td>12</td>
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<td>4</td>
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<tr>
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**Table 2.** Clinical Scores Based on Observations after Single Intracameral Injections

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<th>Limbal Injection</th>
<th>Iritis</th>
<th>Cornea Clarity</th>
<th>Hypopyon</th>
<th>Fibrin Production</th>
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* 0.7% dead cells in one sample; corneal bank standards usually tolerate <2% dead cells for clinical use of human corneas.

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**Table 3.** Corneal Endothelial Cell Counts and Observations with V-MPLs

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<tr>
<th>Sample</th>
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<td>3356</td>
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<td>24</td>
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<td>48</td>
<td>3145</td>
<td>312</td>
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<tr>
<td>37–42</td>
<td>72</td>
<td>3450</td>
<td>280</td>
<td>No</td>
</tr>
</tbody>
</table>
fenses. The rabbits showed no signs of pain or aggressiveness when treated with buprenorphine during this period.

By contrast, bacterial colony counts in rabbits treated with V-MPLs decreased dramatically at 6 hours, and no CFUs were detected on IOL surfaces after 24 hours or in aspirates after 72 hours. Table 4 shows the results of bacterial colony counts for each group at the predetermined intervals.

Preliminary in vitro data published in 2007 showed that MPLs alone provided no bacterial protection.17 Finally, rabbits treated with V-MPLs did not present any sign of infection, in contrast with rabbits without antibiotic.

DISCUSSION

In a prior study, we reported an in vitro study establishing a vancomycin half-life of 2 hours in the anterior chamber of the human eye that enabled us to predict intracameral vancomycin concentrations after intracameral injection of V-MPLs.18 We subsequently demonstrated that our particular MPLs could maintain intracameral vancomycin concentrations at an effective level for at least 24 hours.17 For our present experiments, we needed to know the vancomycin half-life in rabbits to understand variations of intraocular vancomycin concentrations in this species. As no data were available in the literature, we measured declining vancomycin concentrations in rabbit eyes after a single injection and calculated the half-life with a standard elimination formula.

We found that vancomycin was cleared more than four times faster than in humans and that MPLs, as expected, were unable to stabilize the vancomycin concentration for 24 hours. However, as 12 half-lives in rabbits corresponds to 24 hours in humans, the vancomycin concentrations would be sufficient for complete bactericidal action in the latter.15 In rabbits, it would be necessary to quadruple the V-MPL dose to give 24-hour coverage. The primary reason for vancomycin’s decline in rabbit eyes is that elimination is faster than release. In fact, as we demonstrated previously, at least two kinetic phases affects vancomycin release from our MPLs.17 The first phase lasts 1 to 3 hours and is a very fast release of immediately available vancomycin (burst effect), either adsorbed on the MPL surface or present in the outermost layers of the matrix. The second phase lasts an additional 21 hours. The first period in rabbits would represent an equilibrium between release and elimination, with a dramatic concentration decline to 0 between 12 and 24 hours.

**FIGURE 3.** Rabbit corneal endothelium observations after intracameral injections of 0.05 mL V-MPLs suspension (1 mg/mL).
FIGURE 4. Iris and retinal histologic observations (A-H) after intracameral injections of 0.05 mL V-MPLs suspension (1 mg/mL) compared with MPLs, alone (I). (A) The iris 1 hour after injection of V-MPLs showing no inflammatory infiltrate. No inflammation: score –. (B) Iris 3 hours after injection of V-MPLs showing few neutrophils within the stroma. Slight inflammation, score +. (C) Iris 6 hours after injection of V-MPLs showing more abundant inflammatory infiltrate with edema and neutrophils within the stroma. Mild inflammation, score ++. (D) Iris 12 hours after injection of V-MPLs showing edema and neutrophils in a fibrinous exudate within the stroma. Severe acute inflammation, score ++++. (E) Retina 12 hours after injection of V-MPLs showing no inflammation or disorganization. No inflammation, score –. (F) Iris 24 hours after injection of V-MPLs showing fibrinous exudates containing neutrophils at the surface. Severe acute inflammation, score ++++. (G) Iris 48 hours after injection of V-MPLs showing few neutrophils within the stroma. Slight inflammation, score +. (H) Iris 72 hours after injection of V-MPLs showing an absence of inflammatory cells. No inflammation, score –. (I) Iris 12 hours after injection of MPLs showing many neutrophils in the stroma. Severe acute inflammation, score ++++. Hematoxylin-eosin. Magnification: (A–D, F–I) ×200; (E) ×100.
In a recent study Murphy et al.\textsuperscript{20} showed that 26 hours after an intracameral bolus injection of vancomycin (1 mg/0.1 mL), the aqueous humor concentration was four times the MIC. However, as Yoeruek et al.\textsuperscript{21} demonstrated, such a high concentration appears to be toxic to endothelial cells, whereas vancomycin concentrations below 5 mg/mL caused no significant decrease in cell viability, as found in the present study. The results support our present clinical observations on corneal clarity. We therefore conclude that our MPLs, both V-MPLs and MPLs alone, caused no significant corneal toxicity.

Clinical and histologic observations showed that injections of V-MPLs and MPLs had no effect on posterior eye structures. Both treatments, however, induced an intense clinical and histologic inflammatory reaction in the anterior chamber, conjunctiva, and eyelids. Inflammation started soon after injection, but disappeared spontaneously 72 hours later, even though MPLs were still present in the anterior chamber. It seems that either the eye became accustomed to the MPLs after prolonged contact or that aqueous humor clearance reduced the number of MPLs sufficiently to reduce inflammation. A complete degradation of MPLs in the eye may take from 7 days to 8 weeks, depending on the polymer’s molecular weight.\textsuperscript{18} The residual amounts of solvent in the MPLs are themselves too small to induce an inflammatory reaction. However, polymer degradation leads to the formation of polyactic and glycolic acids that could decrease intraocular pH and enhance an inflammatory reaction. The addition of a buffer to the MPL formulation may minimize inflammation and will be analyzed in a further study. Hence, it is essential to evaluate the long-term ocular safety of these particles, especially as in 1999, during extracapsular lens extraction, Axer-Sieger et al.\textsuperscript{12} reported a 20% rate of cystoid macular edema 4 months after use of vancomycin in the irrigating solution during cataract surgery.

In the MPL prophylaxis experiment 4 we found no sign of infection in the V-MPL group at any time interval, validating vancomycin prophylaxis and confirming our previously published findings.\textsuperscript{15} In addition, we demonstrated that the antiadhesion properties of vancomycin needed at least 6 hours to become effective and the bactericidal properties at least 11 hours, with 24 to 48 hours needed for complete bacterial and antiadhesion effects.

In the present report we describe continuing studies of our V-MPLs in rabbits, with promising results as to their possible use in humans. We demonstrate suitable in vivo release and confirm that our V-MPLs help to prevent experimental endophthalmitis. Also, bacterial counts in rabbits with highly infected intraocular lens implants treated simultaneously with V-MPLs showed significant decreases in \textit{S. epidermidis} CFU within the anterior chamber and on IOL surfaces.

\section*{References}

1. Kowalski RP, Romanowski EG, Mah FS, Yates KA, Gordon YJ. Intracameral Vigamox (moxifloxacin 0.5%) is nontoxic and effective in preventing endophthalmitis in a rabbit model. \textit{Am J Ophthalmol.} 2005;140:497–504.


\begin{table}
\caption{Bacterial Colony Counts on Infected IOLs Implanted into the Anterior Chamber of Phakic Rabbits Concomitantly Administered V-MPLs or No Antibiotic}
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
\textbf{Time (h)} & \multicolumn{2}{c|}{\textbf{No Antibiotic}} & \multicolumn{2}{c|}{\textbf{Vancomycin Microparticles}} \\
& \textbf{Aqueous Humor Colonies (CFU/mL)} & \textbf{SD} & \textbf{IOL Colonies (CFU/IOL)} & \textbf{SD} & \textbf{Aqueous Humor Colonies (CFU/mL)} & \textbf{SD} & \textbf{IOL Colonies (CFU/IOL)} & \textbf{SD} \\
\hline
6 & 9,270 & 1,356 & \textbf{4.7} \times \textbf{10}^5 & \textbf{2.1} \times \textbf{10}^5 & 513 & 122 & \textbf{660} & \textbf{142} \\
24 & 7,730 & 2,534 & \textbf{3.9} \times \textbf{10}^5 & \textbf{3.0} \times \textbf{10}^5 & 67 & 46 & 0 & 0 \\
72 & 11,390 & 1,100 & \textbf{5.2} \times \textbf{10}^5 & \textbf{1.1} \times \textbf{10}^5 & 0 & 0 & 0 & 0 \\
168 & 9,740 & 3,244 & \textbf{3.2} \times \textbf{10}^5 & \textbf{0.9} \times \textbf{10}^5 & 0 & 0 & 0 & 0 \\
\hline
\end{tabular}
\end{table}


