The Safety of Intraocular Linezolid in Rabbits

Sara L. Duke,[1] *Leila I. Kump,*[1] *Yang Yuan,*[2] *William W. West,*[3] *Andrew J. Sachs,*[2] *Neena B. Haider,*[2] *and Eyal Margalit*[1]

Purpose. Intraocular injection of linezolid, a synthetic oxazolidinone antibiotic, was performed in rabbits to assess its safety as a possible treatment for endophthalmitis.

Methods. Linezolid, 300 μg/0.1 mL, 200 μg/0.1 mL, or 100 μg/0.1 mL, was injected into the vitreous of the right eye of 12 rabbits. Balanced saline solution was injection into the left eye of each rabbit as a control. A standard electroretinogram (ERG) was obtained before injection and repeated 2 days and 1 and 4 weeks after injection. Intraocular pressure (IOP) was also measured after injection. After the experiment, the rabbits were euthanatized and the retinas were examined by light and electron microscopy. Differences between the two eyes in the ERGs, IOP, and histopathology were recorded.

Results. There were no statistically significant differences in the electroretinograms obtained between the linezolid-injected eyes and the control eyes. Histopathology showed no changes in the study eyes compared with the control eyes.

Conclusions. Preservative-free linezolid is nontoxic to the retinas of rabbits when injected intravitreally, and this route can therefore be considered for the administration of linezolid in the treatment of endophthalmitis. (*Invest Ophthalmol Vis Sci.* 2010;51:3115–3119) DOI:10.1167/iovs.09-4244

Bacterial endophthalmitis is a rare but serious complication of intraocular surgery that may result in significant vision loss. Postoperative and posttrauma cases of endophthalmitis are most frequently due to infections with Gram-positive bacteria such as coagulase-negative *Staphylococcus*, *Streptococcus viridans*, *Staphylococcus aureus*, *Propionibacterium acnes*, and *Enterococcus* species.[1] Because of the high incidence of severe vision loss in endophthalmitis, several techniques have been adopted to reduce the risk of infection. These include preoperative application of topical antibiotics and povidone-iodine, addition of antibiotics to the intravenous solution, and postoperative application of topical antibiotics.[2]

Linezolid is a synthetic oxazolidinone antibiotic that is both chemically and functionally distinct.[3] It acts by preventing the initiation phase of protein synthesis, whereas other antibiotics target subsequent phases, and so cross-resistance is unlikely.[4] Linezolid is highly active against nearly all Gram-positive bacteria including methicillin-resistant *Staphylococcus aureus*.[5] Harper reported linezolid susceptibility in 100% of the coagulase-negative *Staphylococcus* endophthalmitis isolates.[6] Linezolid is also used to treat infections caused by vancomycin-resistant *Enterococcus faecium* and *E. faecalis* (VRE).[7] Previous studies have focused on intraocular concentrations of linezolid after systemic administration; however, bacterial endophthalmitis is primarily treated by intraocular inoculation of antibiotics because of poor transmission across the blood–ocular barrier. Thus, we devised an experiment to determine whether linezolid is nontoxic to the retina when injected into the vitreous of rabbits.

Methods

All animals (pigmented Dutch-belted rabbits) were used according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the University of Nebraska Medical Center guidelines for the use of animals in experimental procedures. All surgical and examination procedures were performed on rabbits under anesthesia induced with an intramuscular injection of 35 mg/kg of body weight of ketamine hydrochloride and 5 mg/kg of body weight of xylazine hydrochloride (both from Phoenix Scientific, Inc., St. Joseph, MO). Before all intravitreal injections, the eyes were cleaned with a few drops of 5% povidone-iodine solution.

To examine drug toxicity in vivo, we injected preservative-free linezolid (Zyvox; Pfizer, New York, NY) into 12 rabbits' right eyes. One of three different concentrations of linezolid, 300 μg/0.1 mL, 200 μg/0.1 mL, or 100 μg/0.1 mL, was injected into the vitreous cavity of the right eye in four rabbits per dose. Balanced saline solution (0.1 mL; Alcon, Fort Worth, TX) was injected into the vitreous cavity of the left eye in the same animals. The vitreous cavity was entered through the superotemporal sclera less than 1 mm posterior to the limbus with a 27-gauge needle connected to a 1-mL syringe that contained 0.1 mL of different concentrations of linezolid (experimental eyes) or 0.1 mL of balanced saline (control eyes). After injection of linezolid, the eye was rinsed with povidone-iodine again, and 0.1 mL of aqueous humor was withdrawn from the anterior chamber via paracentesis performed by insertion into the limbus at 12 o'clock of a 30-gauge needle connected to a 1-mL syringe.

The eyes were evaluated by anterior and posterior biomicroscopy, indirect ophthalmoscopy and electroretinography (ERG) before the injection and at days 2, 7, 14, 21, and 28 after injection.

ERGs were performed as previously described.[8] Briefly, the pupils were dilated with 1 drop of tropicamide 1% and phenylephrine hydrochloride 2.5% (both from Bausch & Lomb Pharmaceuticals, Inc., Tampa, FL). The animals were dark adapted for 30 minutes. The ERG setup consisted of a contact lens electrode for each eye, a reference needle electrode positioned at the lateral canthus, and a ground disc electrode that was placed in the mouth of the animals. Standard ERGs were recorded (UTAS E4000 System; LKC Technologies, Gaithersburg, MD). An average of three separate ERGs was used at each time point for each eye.

Statistical analyses of the data were performed by using the method of generalized estimating equations (GEEs), to account for the repeated measurements within subjects. This approach characterizes the average response in eyes measured at the same time point as a function of

From the Departments of [1]Ophthalmology and Visual Sciences, [2]Genetics Cell Biology and Anatomy, and [3]Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska.

Supported in part by an unrestricted grant from Research to Prevent Blindness.

Submitted for publication June 30, 2009; revised November 30, 2009; accepted December 15, 2009.

Disclosure: **S.L. Duke**, None; **L.I. Kump**, None; **Y. Yuan**, None; **W.W. West**, None; **A.J. Sachs**, None; **N.B. Haider**, None; **E. Margalit**, None

Corresponding author: Eyal Margalit, Retina Service, Director, Department of Ophthalmology and Visual Sciences, 985540 Nebraska Medical Center, Omaha, NE 68198-5540; emargalit@unmc.edu.

TABLE 1. Descriptive Statistics for Each Eye in Rabbits Treated with 300 μg Linezolid/0.1 mL in the Right Eye and Balanced Saline in the Left Eye

	Preoperative		Day 2		Week 1		Week 2		Week 3		Week 4	
	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD	OS
Scotopic												
Median	102.52	114.09	90.96	111.29	110.54	99.42	136.67	139.06	124.29	104.23	102.76	109.19
Min	75.94	76.47	53.73	69.71	87.74	74.90	67.70	80.37	88.46	88.19	97.81	96.16
Max	156.47	150.06	126.13	116.60	186.73	195.29	159.43	148.47	190.76	130.97	115.43	129.46
Mean	109.36	113.68	90.44	102.22	123.89	117.26	125.12	126.74	131.95	106.90	104.69	111.00
SEM	17.09	15.59	16.17	10.93	21.69	27.50	21.19	15.62	21.40	9.19	4.24	7.34
Photopic												
Median	44.28	40.72	25.92	30.93	51.53	46.05	55.28	52.02	38.94	39.53	44.10	40.21
Min	30.73	32.68	11.78	26.02	48.22	23.38	44.72	40.27	35.48	34.33	31.97	27.97
Max	57.72	56.38	39.45	57.13	93.42	80.25	85.88	73.85	82.58	54.50	52.55	49.40
Mean	44.25	42.63	25.77	36.25	61.17	48.93	60.29	54.54	48.99	41.97	43.18	39.20
SEM	5.98	4.97	5.74	7.20	10.78	11.76	9.87	7.01	11.23	4.79	4.60	4.21

The scotopic and photopic b-wave amplitudes are presented in μV. Scotopic, the stimulus was a scotopic white flash 0 dB. Photopic, the stimulus was a single flash photopic ERG. OD, right eye; OS, left eye.

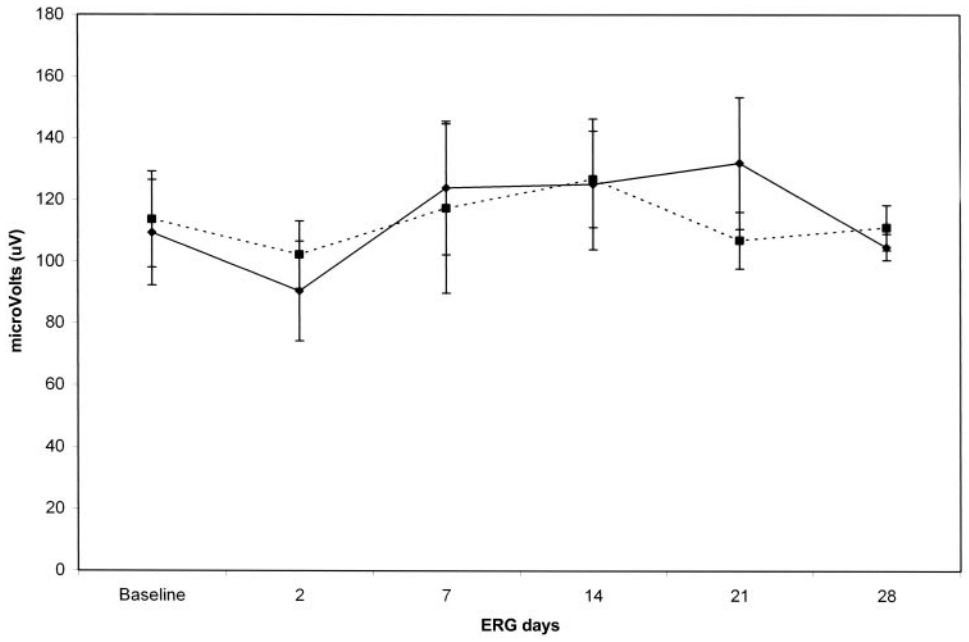

FIGURE 1. The mean amplitude of the ERG b-wave (in microvolts), as a function of time, after exposure of rabbits to a 0-dB white-flash light stimulus after injection with 300 μg linezolid/0.1 mL in the right eye (*solid line*) and balanced saline in the left eye (*dashed line*).

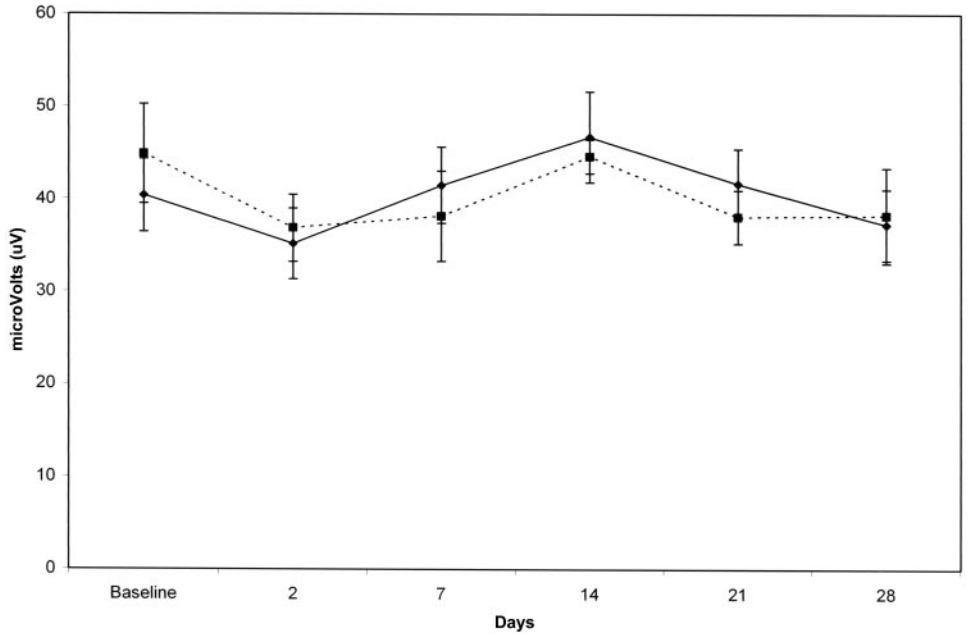

FIGURE 2. The mean amplitude of the ERG a-wave (in microvolts), as a function of time, after exposure of rabbits to a 0-dB white-flash light stimulus after injection with 300 μg linezolid/0.1 mL in the right eye (*solid line*) and balanced saline in the left eye (*dashed line*).

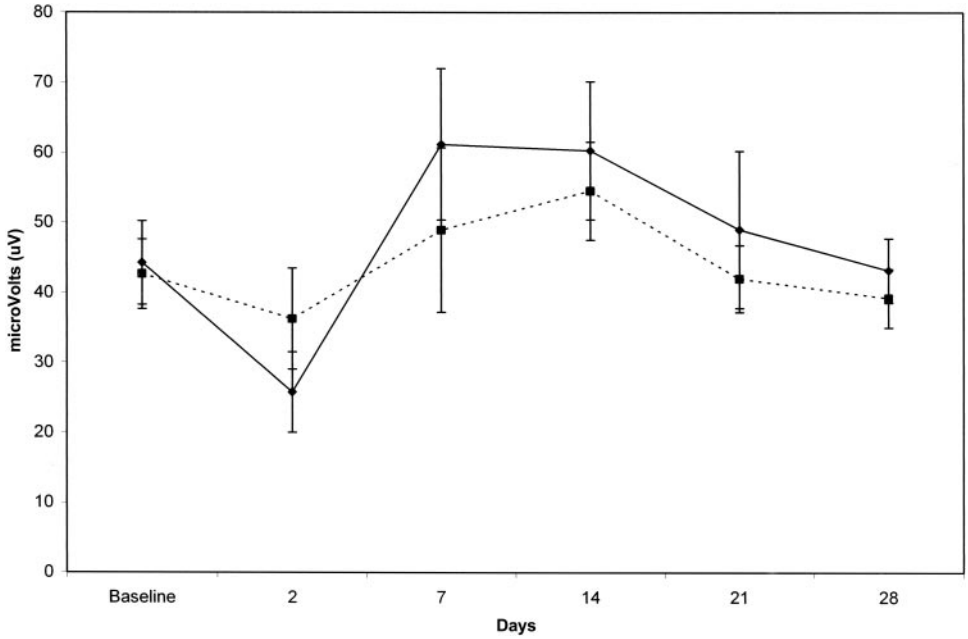

FIGURE 3. The mean amplitude of the ERG b-wave (in microvolts), as a function of time, after a single-flash photopic ERG was performed on all rabbits injected with 300 μg linezolid/0.1 mL in the right eye (*solid line*) and balanced saline in the left eye (*dashed line*).

treatment and provides robust estimates of the standard errors of the model parameters.

After the injection, we measured intraocular pressure (IOP) in both eyes in all rabbits with a pneumotonometer (model 30 Classic; Mentor, Norwell, MA).

After the experiment, the rabbits were euthanatized with an overdose injection of phenobarbital via the ear vein. The eyes were immediately enucleated and fixed in a solution of formalin 10% for light microscopy or in a glutamate-formalin mixture (glutaraldehyde 2%, paraformaldehyde 2%, acrolein 0.5%, and Sorenson's phosphate buffer 0.1 M [pH 7.4]) for electron microscopy examination.

RESULTS

The GEE regression model described herein was used to assess the ERG results. There was no evidence that the b-wave amplitude produced after white light stimulation (0 dB) differed significantly in the eyes treated with 300 μg linezolid/0.1 mL compared with balanced saline–treated eyes ($P = 0.86$, two tails, Student's *t*-test). Similarly, the b-wave response amplitudes produced after a single-flash photopic ERG did not differ significantly between the eyes treated with 300 μg linezolid/0.1 mL compared with the balanced saline–treated eyes ($P = 0.59$). Descriptive statistics for each eye at each time point are provided in Table 1 and Figures 1, 2, 3, and 4. Descriptive statistics for the difference in the response (right eye minus left eye) are provided in Table 2.

The baseline IOP for pigmented rabbits, as measured with a pneumatonometer, was 24.5 ± 1.7 mm Hg.[9] The mean IOPs in the eyes treated with 300 μg linezolid/0.1 mL at 2 and 5 weeks after injection were 17.1 ± 1.3 and 20.4 ± 2.6 mm Hg, respectively. The mean IOPs for untreated eyes from the same

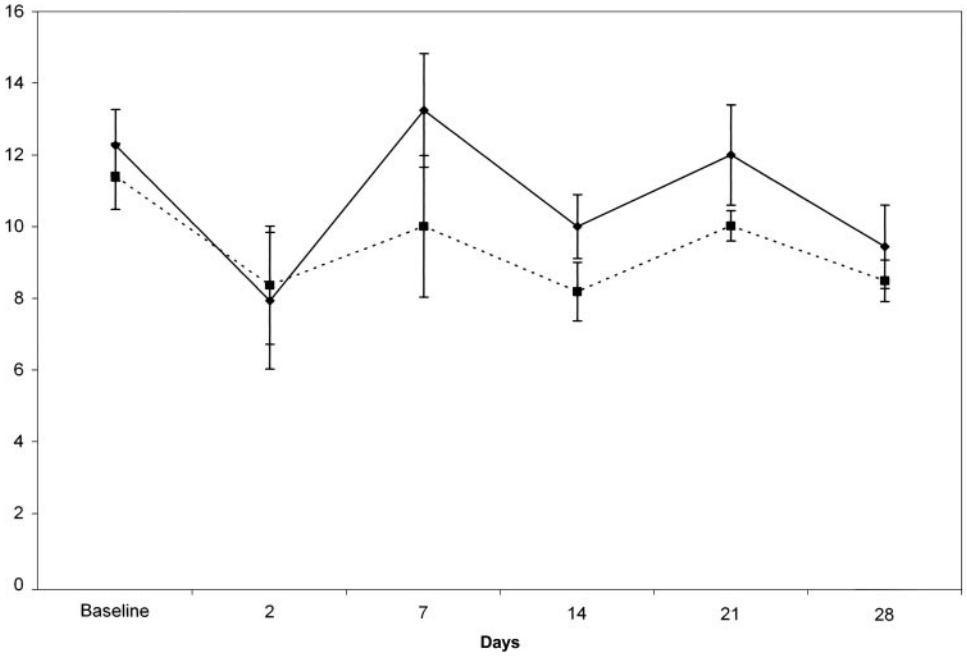

FIGURE 4. The mean amplitude of the ERG a-wave (in microvolts), as a function of time, after a single-flash photopic ERG was performed on all rabbits injected with 300 μg linezolid/0.1 mL in the right eye (*solid line*) and balanced saline in the left eye (*dashed line*).

TABLE 2. Descriptive Statistics for the Difference between the Eyes (Right Eye Minus Left Eye) in Rabbits Treated with 300 μg Linezolid/0.1 mL in the Right Eye and Balanced Saline in the Left Eye

	Preoperative Difference	Day 2 Difference	Week 1 Difference	Week 2 Difference	Week 3 Difference	Week 4 Difference
Scotopic						
Median	−11.57	−20.33	11.11	−2.39	20.06	−6.43
Min	−0.53	−15.99	12.84	−12.67	0.27	1.66
Max	6.41	9.53	−8.56	10.96	59.79	−14.03
Mean	−4.31	−11.78	6.63	−1.63	25.04	−6.31
SEM	1.50	5.24	−5.81	5.58	12.21	−3.10
Photopic						
Median	3.57	−5.02	5.48	1.50	−0.58	3.89
Min	−1.95	−14.23	24.83	−11.20	1.15	4.00
Max	1.33	−17.68	13.17	13.50	28.08	4.15
Mean	1.63	−10.49	5.75	1.56	7.02	3.98
SEM	1.01	−1.47	2.86	3.68	6.44	0.39

The scotopic and photopic b-wave amplitudes are presented in microvolts. Scotopic, white flash stimulus, 0 dB; photopic, a single-flash photopic ERG.

group were 15.5 ± 1.7 and 21.6 ± 2.4 mm Hg at 2 and 5 weeks after injection, respectively. There was a tendency for lower IOP in both control and study eyes at 2 weeks after injection.

Histopathology specimens were examined by light microscopy and electron microscopy. There were no apparent differences in histology between the eyes injected with linezolid and the eyes injected with balanced saline. Figure 5 shows the light microscopy image of an eye injected with 300 μg linezolid/0.1 mL and Figure 6 shows a control eye. Figure 7 shows an electron microscopy image of an eye injected with 300 μg linezolid/0.1 mL.

DISCUSSION

In this study, we examined the safety profile of intraocular linezolid. The drug is commercially available for use in oral and intravenous formulations. It has commonly been used to treat infections caused by VRE, and it has a low minimum inhibitory concentration (MIC) against virtually all Gram-positive bacteria.

Antibiotics have been used successfully to treat various ocular infectious for numerous years, but the treatment of diseases such as bacterial endophthalmitis has remained difficult. Many cases continue to have poor outcomes and so physicians use intravitreal antibiotics for better results. However, some of these antibiotics have been shown to have adverse ocular effects.

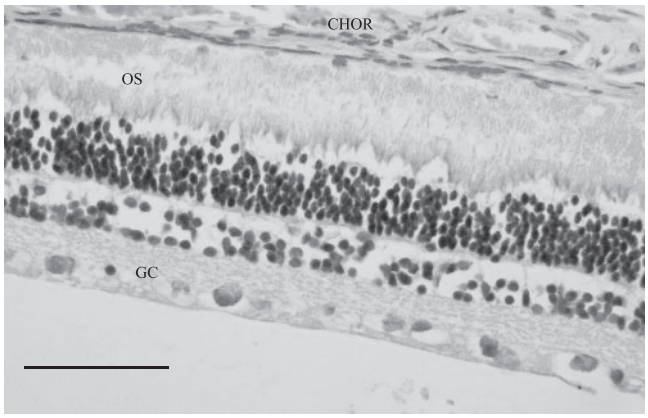

FIGURE 5. Light microscopy of an eye injected with 300 μg linezolid/0.1 mL. GC, ganglion cell layer; OS, outer segments; CHOR, choroid. H&E stain; magnification, ×400. Bar, 50 μm.

It has been well documented that intravitreal aminoglycoside injection may cause macular infarction, even at low doses such as 0.1 mg gentamicin or 0.2 mg amikacin.[10] Hancock reported that concentrations of 10 mg/mL of gentamicin have neurotoxic effects.[11] In another study, the authors observed retinal toxicity in the form of ERG and histologic changes in

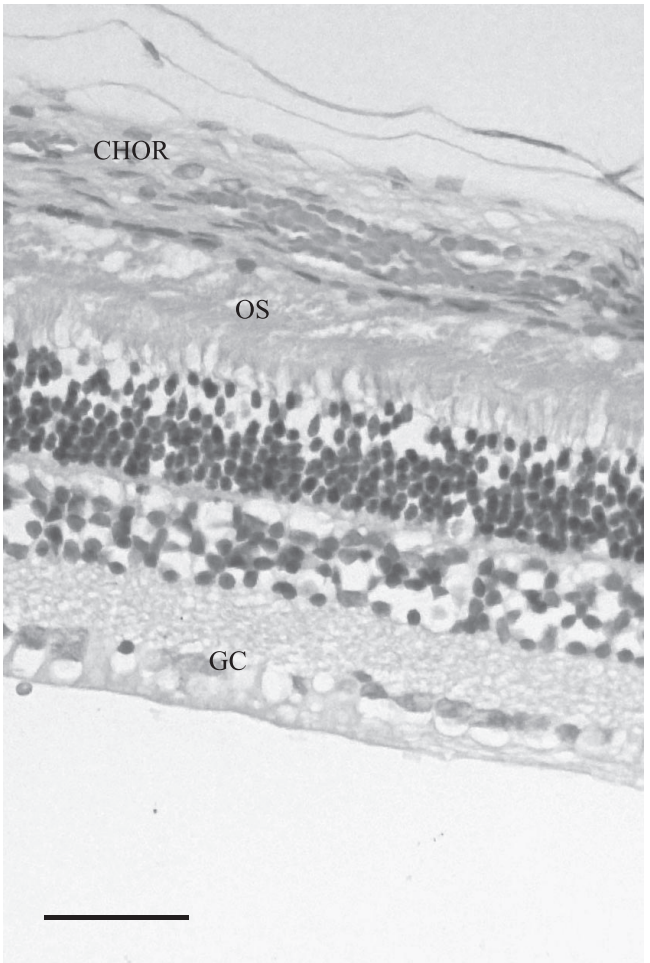

FIGURE 6. Light microscopy of an eye injected with balanced saline. H&E stain; magnification, ×400. Bar, 50 μm.

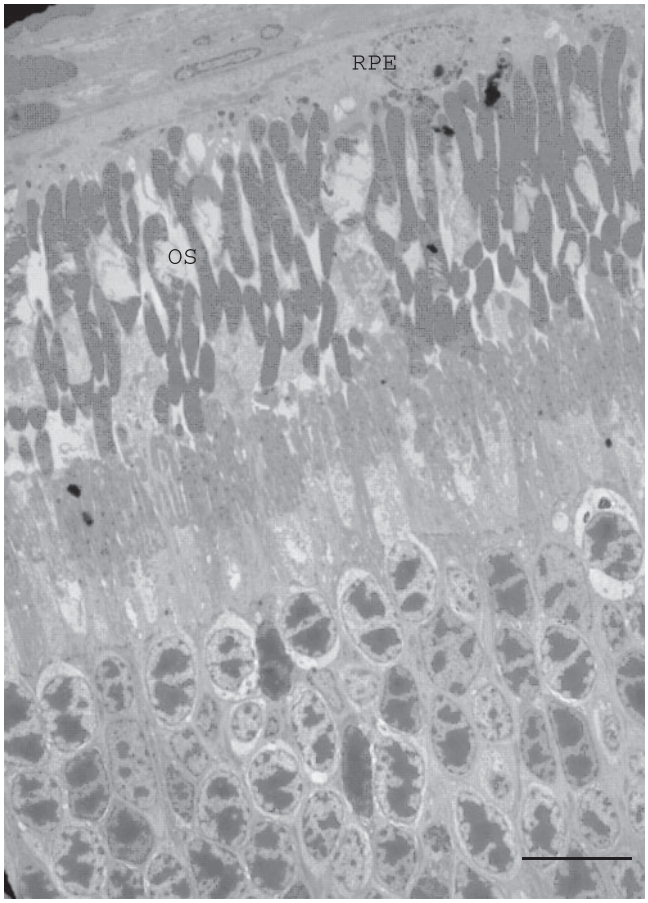

FIGURE 7. Electron microscopy of an eye injected with 300 μg linezolid/0.1 mL. RPE, retinal pigment epithelium. Magnification, ×2230. Bar, 10 μm.

eyes that were injected with doxycycline at concentrations of 250 μg/0.1 mL or greater.[12]

Because of the broad spectrum of efficacy and potency of linezolid, it has been studied as a potential therapy for bacterial endophthalmitis. We used an injection of 300 μg/0.1 mL linezolid that was not toxic to the retina or lens. Previous studies assessing the intraocular concentration of linezolid after oral or intravenous administration reported maximum concentrations of 6.8 μg/mL in the aqueous humor and 5.7 μg/mL in the vitreous.[13–15]

Our study demonstrated the safety of intravitreal injections of linezolid in rabbits, both functionally and histologically. The major side effects of intravitreal antibiotics such as gentamicin and doxycycline were not observed in this study, though the duration of the follow-up was relatively short. In conclusion, our data show that linezolid, an oxazolidinone antibiotic, is nontoxic to the retina when injected into the vitreous of the rabbit.

References

1. Benz MS, Scott IU, Flynn HW, Unonius N, Miller D. Endophthalmitis isolates and antibiotic sensitivities: a 6-year review of culture-proven cases. *Am J Ophthalmol.* 2004;137:38–42.
2. Riordan-Eva P, Whitcher JP. *Vaughan and Asbury's General Ophthalmology.* 17th ed. New York: McGraw-Hill Medical; 2008:395.
3. Fung HB, Kirschenbaum HL, Ojofeitimi BO. Linezolid: an oxazolidinone antimicrobial agent. *Clin Ther.* 2001;23:356–391.
4. Ament PW, Jamshed N, Horne JP. Linezolid: its role in the treatment of gram-positive, drug-resistant bacterial infections. *Am Fam Phys.* 2002;65:663–670.
5. Wunderink RG, Rello J, Cammarata SK, Croos-Dabrera RV, Kollef MH. Linezolid vs. vancomycin: analysis of two double-blind studies of patients with methicillin-resistant Staphylococcus aureus nosocomial pneumonia. *Chest.* 2003;124:1789–1797.
6. Harper T, Miller D, Flynn HW Jr. In vitro efficacy and pharmacodynamic indices for antibiotics against coagulase-negative staphylococcus endophthalmitis isolates. *Ophthalmology.* 2007;114:871–875.
7. Diekema DJ, Jones RN. Oxazolidinone antibiotics. *Lancet.* 2001;358:1975–1982.
8. Nystuen AM, Sachs AJ, Yuan Y, Heuermann L, Haider NB. A novel mutation in Prph2, a gene regulated by Nr2e3, causes retinal degeneration and outer-segment defects similar to Nr2e3 (rd7/rd7) retinas. *Mamm Genome.* 2008;19:623–633.
9. Chu TC, He Q, Potter DE. Biodegradable calcium phosphate nanoparticles as a new vehicle for delivery of a potential ocular hypotensive agent. *J Ocul Pharmacol Ther.* 2002;18:507–514.
10. Campichiaro PA, Conway BP. Aminoglycoside toxicity: a survey of retinal specialists—implications for ocular use. *Arch Ophthalmol.* 1991;109:946–950.
11. Hancock HA, Guidry C, Read RW, et al. Acute aminoglycoside retinal toxicity in vivo and in vitro. *Invest Ophthalmol Vis Sci.* 2005;46:4804–4808.
12. Aydin E, Kazi AA, Peyman GA, et al. Retinal toxicity of intravitreal doxycycline: a pilot study (in Spanish). *Arch Soc Esp Oftalmol.* 2007;82:223–228.
13. Prydal JI, Jenkins DR, Lovering A, Watts A. The pharmacokinetics of linezolid in the non-inflamed human eye. *Br J Ophthalmol.* 2005;89:1418–1419.
14. Fiscella RG, Lai WW, Buerk B, et al. Aqueous and vitreous penetration of linezolid (Zyvox) after oral administration. *Ophthalmology.* 2004;111:1191–1195.
15. Horcajada JP, Atienza R, Sarasa M, Soy D, Adan A, Mensa J. Pharmacokinetics of linezolid in human non-inflamed vitreous after systemic administration. *J Antimicrob Chemother.* 2009;63:550–552.