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ORIGINAL ARTICLE

Safety of intravitreal linezolid injection: electroretinographic and histopathologic studies in rabbits

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ABSTRACT

Objective

This study evaluated the safety of linezolid as an alternative intravitreal drug for the treatment of bacterial endophthalmitis.

Methods

Eight albino rabbits were divided randomly into 2 equal groups: the right eyes were injected intravitreally with linezolid (100 mcg/0.10mL in Group 1 and 200 mcg/0.10mL in Group 2) and the left eyes were injected with 0.10mL balanced salt solution. Indirect ophthalmoscopy before and after intravitreal injections determined the presence of any precipitates in the vitreous. Electroretinography (ERG) and histopathology evaluated the effects in the retina.

Results

No vitreous precipitates were found in all groups. Scotopic ERG showed a decrease in b-wave amplitude ($p < 0.05$) in Group 2 between 3 hours and 2 days and between 3 hours and 7 days after injection. Histopathology showed minimal inflammatory cells (<3 cells/hpf) in Group 2 and controls, trace vacuolizations in the ganglion-cell layer and partial loss of photoreceptor outer segment in Group 1 and in controls, and minimal decrease in the outer-nuclear-cell density in all groups.

Conclusion

Intravitreal injection of up to 200 mcg linezolid is safe and well tolerated in rabbit eyes and may be used in the treatment of human bacterial endophthalmitis following further studies.

Keywords: *Endophthalmitis, Intravitreal drug, Linezolid, Electroretinography, Retinal toxicity*

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BACTERIAL endophthalmitis is an intraocular infection that frequently results in loss of vision. Early treatment and rapid elimination of the infecting organisms are vital to the preservation of vision. Since systemic antibiotics do not achieve significant vitreous concentrations, intravitreal administration remains a key strategy in the clinical management of this condition.

The Endophthalmitis Vitrectomy Study (EVS) has recommended a combination of vancomycin plus either amikacin, ceftazidime, or ciprofloxacin, which all have activity against Gram-negative bacilli, as initial treatment for bacterial endophthalmitis.[1] Vancomycin is considered the drug of choice for a range of infections and is nontoxic at a dose of 1.0 mg in 0.1 mL.[2] Aminoglycosides, such as amikacin or gentamicin, are often used but intravitreal injection of gentamicin has been reported to cause macular toxicity,[3] and the risk also extends to amikacin even though animal experiments have shown it to be safer than gentamicin.[4-5] Ceftazidime, in contrast, carries a lower risk of retinal toxicity and has a broader therapeutic index,[6-7] but it precipitates in the vitreous humor at body temperature.[6]

The emergence of vancomycin-resistant *Enterococci* species and the potential for macular toxicity of the other intravitreal drugs emphasize the need for alternative drugs for intravitreal injections. Linezolid is a synthetic antibacterial agent belonging to a new class of antibiotics, the oxazolidinones, which is indicated for infections caused by aerobic Gram-positive bacteria. The *in vitro* spectrum of activity also includes certain Gram-negative and anaerobic bacteria. Linezolid inhibits bacterial protein synthesis through a mechanism of action different from that of other antibacterial agents; therefore, cross-resistance with other classes of antibiotics is unlikely. It binds to a site on the bacterial 23S ribosomal RNA of the 50S subunit and prevents the formation of a functional 70S initiation complex, an essential component of the bacterial-translation process.[8]

In vitro studies have demonstrated additivity or indifference between linezolid and vancomycin, gentamicin, rifampin, imipenem–cilastatin, aztreonam, ampicillin, or streptomycin antimicrobial activity.[8] Linezolid has been shown to be active against most isolates of aerobic and facultative Gram-positive microorganisms including *Enterococcus faecium* (including vancomycin-resistant strains), *Staphylococcus aureus* (including methicillin-resistant strains), *Enterococcus faecalis* (including vancomycin-resistant strains), *Staphylococcus epidermidis* (including methicillin-resistant strains), *Streptococcus pneumoniae* (including multidrug-resistant isolates, MDRSP); and Gram-negative microorganisms.[9-10]

This study evaluated the safety of intravitreal injection of linezolid (Zyvox, Pfizer, New York, NY, USA) in albino rabbits based on the following parameters: precipitates in the vitreous, effect on electroretinography (ERG), and any inflammatory or necrotic changes in the retina.

METHODOLOGY

Eight albino rabbits were divided randomly into 2 equal groups. Group 1 received 100 mcg/0.10 mL and Group 2 received 200 mcg/0.10 mL. They were handled according to the tenets of Association for Research in Vision and Ophthalmology in relation to animal research.

The rabbits were anesthetized with intramuscular injection of ketamine (Ketaject, PharmAsia-Cuvest, Makati City, Philippines) at a dose of 65 mg/kg and 2 drops of 0.5% proparacaine hydrochloride (Alcaine, Alcon Laboratories, Fort Worth, TX, USA). Ciprofloxacin ophthalmic solution was applied to the eyes before injection and tobramycin ophthalmic ointment after injection.

The right eyes of the rabbits received intravitreal linezolid and the left eyes 0.10 mL balanced salt solution. Intravitreal injection with a gauge 27 needle on tuberculin syringe was given 2 millimeters posterior to the temporal limbus. The needle was angled toward the optic nerve until the tip was visible in the center of the vitreous, approximately 3 millimeters in depth.

All eyes were examined 1 day before intravitreal linezolid injection, 3 hours after injecton, and on day 2 and day 7 postinjection. Pupils were dilated with 0.5% tropicamide and 2.5% phenylephrine for indirect ophthalmoscopy to check for vitreous precipitates, opacities, and hemorrhages.

Prior to ERG (Nihon Kohden Neuropack), the rabbits were kept in a dark room for 20 minutes. The recording electrode was placed on the lower lid, the reference electrode centrally on the shaven forehead, and the ground electrode on the earlobe. Micropore tapes were placed on the electrodes for stability. ERG consisting of photopic, scotopic, bright flash, and 30-Hertz flicker tests were obtained 3 hours, 2 days, and 7 days postinjection. The implicit time and amplitude of the corresponding wave component of each test were measured.

Color and red-free (green) fundus photos of each eye were also taken after indirect ophthalmoscopy on day 7 postinjection using a Zeiss Visupack FF450 (Zeiss Corporation, Oberkochen, Germany) fundus camera. Afterwards, the rabbits were euthanized with an overdose of intraperineal ketamine. The eyes were enucleated and fixed immediately in 10% formaldehyde. After 48 hours in the fixative, gross examinations of the tissues were performed. The enucleated eyes were trisected parallel to the cornea-optic-nerve axis. Tissues were then embedded in paraffin, sectioned at a thickness of 6 μm, and stained with hematoxylin-eosin. The retinas were

evaluated for inflammatory and necrotic changes by an ocular pathologist blinded to the study regimen. Light microscopy was used for examination of the retina.

ERG data were entered into Microsoft Excel (Microsoft Corp., Redwood, WA, USA) for statistical analysis. The implicit time and amplitude of the corresponding waves produced in photopic, scotopic, bright flash, and 30-Hertz flicker tests of Group 1, Group 2, and control eyes were compared with one another and at different times postinjection using paired *t*-test. A *p*-value of less than or equal to 0.05 was considered statistically significant.

RESULTS

Indirect ophthalmoscopy showed no opacity, precipitation, or hemorrhage in the vitreous in all eyes. Vitreous was clear after linezolid and BSS injection (Figures 1-4).

The characteristic electroretinogram of each test are shown in Figures 5 and 6. In the scotopic test (rod response), a statistically significant decrease in the b-wave amplitude ($p < 0.05$) was noted in Group 2 between 3 hours and 2 days ($p = 0.04$) and between 3 hours and 7 days ($p = 0.04$) after the administration of linezolid, without a concurrent implicit time change. In the photopic, bright flash, and 30-Hertz flicker tests, the implicit time and amplitude of corresponding waves showed no significant change at any dose or at any time after drug administration. Table 1 shows the *p* values of the dose-dependent ERG implicit time and amplitude responses between the different time periods and the *p* values of time-dependent ERG implicit time and

Table 1. ***p* values of dose-dependent and time-dependent electroretinogram (ERG) implicit time and amplitude responses between different time periods and between groups.**

		Dose-dependent				Time-dependent			
Test		**Dose**	**3 hours vs. 2 days**	**3 hours vs. 7 days**	**2 days vs. 7 days**	**Time**	**Control vs. Group1**	**Control vs. Group2**	**Group1 vs. Group2**
Scotopic	B-wave Implicit Time	Control	0.22	0.24	0.43	3 hours	0.09	0.10	0.45
		Group 1	0.34	0.29	0.18	2 days	0.25	0.17	0.37
		Group 2	0.15	0.37	0.16	7 days	0.46	0.47	0.50
	B-wave Amplitude	Control	0.28	0.11	0.22	3 hours	0.37	0.16	0.19
		Group 1	0.50	0.26	0.42	2 days	0.57	0.44	0.10
		Group 2	0.04	0.01	0.29	7 days	0.62	0.21	0.10
Photopic	A-wave Implicit Time	Control	0.38	0.50	0.50	3 hours	0.50	0.20	0.20
		Group 1	0.50	0.30	0.49	2 days	0.38	0.20	0.15
		Group 2	0.50	0.50	0.50	7 days	0.20	0.50	0.20
	A-wave Amplitude	Control	0.30	0.36	0.49	3 hours	0.28	0.06	0.27
		Group 1	0.33	0.26	0.48	2 days	0.07	0.22	0.11
		Group 2	0.05	0.06	0.43	7 days	0.35	0.50	0.34
	B-wave Implicit Time	Control	0.17	0.09	0.50	3 hours	0.42	0.38	0.43
		Group 1	0.41	0.43	0.49	2 days	0.07	0.36	0.21
		Group 2	0.21	0.13	0.41	7 days	0.39	0.43	0.33
	B-wave Amplitude	Control	0.41	0.36	0.37	3 hours	0.38	0.44	0.29
		Group 1	0.48	0.46	0.49	2 days	0.47	0.35	0.37
		Group 2	0.16	0.30	0.44	7 days	0.25	0.44	0.27
Bright Flash	A-wave Implicit Time	Control	0.20	0.20	0.46	3 hours	0.10	0.20	0.06
		Group 1	0.06	0.09	0.47	2 days	0.50	0.20	0.20
		Group 2	0.20	0.20	0.46	7 days	0.24	0.37	0.16
	A-wave Amplitude	Control	0.35	0.47	0.42	3 hours	0.29	0.41	0.15
		Group 1	0.10	0.08	0.37	2 days	0.36	0.45	0.44
		Group 2	0.48	0.13	0.45	7 days	0.13	0.34	0.10
	B-wave Implicit Time	Control	0.46	0.23	0.46	3 hours	0.46	0.30	0.28
		Group 1	0.50	0.35	0.49	2 days	0.50	0.42	0.45
		Group 2	0.24	0.15	0.49	7 days	0.09	0.32	0.51
	B-wave Amplitude	Control	0.27	0.20	0.47	3 hours	0.35	0.27	0.17
		Group 1	0.22	0.14	0.45	2 days	0.37	0.19	0.26
		Group 2	0.19	0.33	0.46	7 days	0.40	0.16	0.40
30-Hertz Flicker	Implicit Time	Control	0.50	0.50	0.50	3 hours	0.29	0.18	0.29
		Group 1	0.37	0.07	0.47	2 days	0.21	0.27	0.34
		Group 2	0.27	0.23	0.45	7 days	0.27	0.50	0.29
	Amplitude	Control	0.48	0.14	0.30	3 hours	0.17	0.14	0.42
		Group 1	0.50	0.50	0.50	2 days	0.20	0.27	0.40
		Group 2	0.33	0.33	0.50	7 days	0.24	0.38	0.14

amplitude responses between the different groups.

Histopathologic findings (Figure 7) included the presence of minimal inflammatory cells (<3 cells/hpf) in the nerve-fiber layer in 2 rabbit eyes in Group 2 and in 1 control eye; vacuolizations in the ganglion-cell layer in 3 eyes in Group 1 and in 2 control eyes; minimal decrease in the cell density in the outer nuclear layer in 2 eyes in Group 1, in 1 eye in Group 2, and in 3 control eyes; and partial loss of photoreceptor outer segment in 2 eyes in Group 1 and in 2 control eyes. The remaining retinal layers did not show any inflammatory or necrotic changes. The anterior segments of all eyes were normal.

DISCUSSION

The data in this experiment suggest linezolid has minimal effects on ERG and few histopathologic changes in the retina. No vitreous precipitates were seen.

ERG is a diagnostic tool used to detect retinal pathology. The two parameters of the ERG that are measured clinically are the amplitude (measured in microvolts, μV) and the implicit time (measured from the onset of the stimulus to the peak of the response in milliseconds). The anatomical and functional similarities between human and rabbit retinas have made the latter appropriate models for studying drug toxicity even though the retinal anatomy of the rabbit is slightly different. For example, the human retina has three types of cone cells whereas rabbit retina has only two.[11, 12] Thus, it is preferable to use time- and dose-dependent ERG changes in a given group to detect any toxicity.

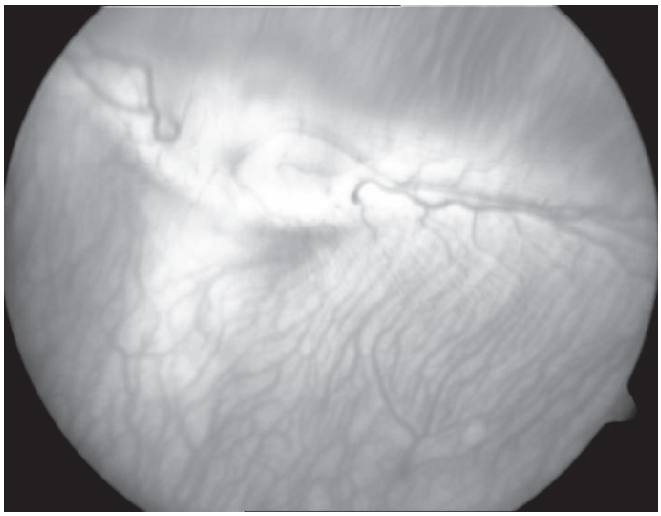


Figure 1. Fundus photo of rabbit eye showing clear media and no vitreous precipitation after intravitreal injection of 200 mcg linezolid.

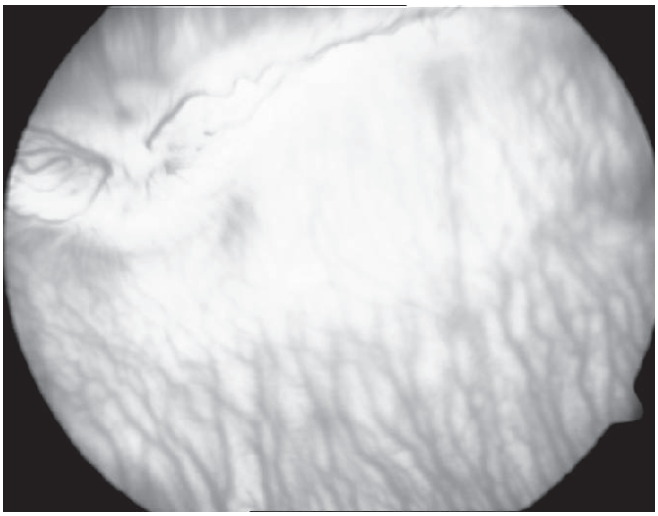


Figure 2. Fundus photo of control rabbit eye showing clear media after intravitreal injection of balanced salt solution.

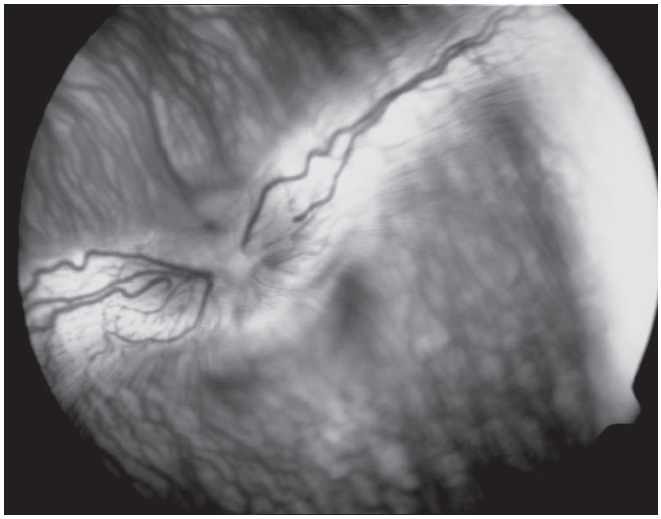


Figure 3. Red-free photo of rabbit eye showing no gross retinal-nerve-fiber-layer changes after intravitreal injection of 200 mcg linezolid.

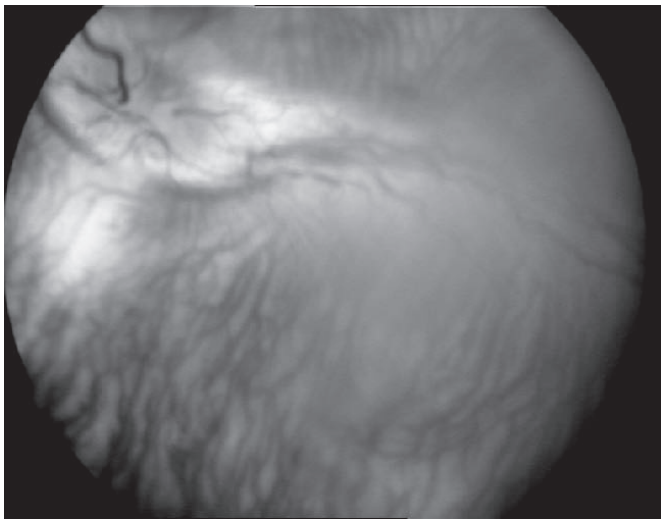


Figure 4. Red-free photo of control rabbit eye showing no gross retinal-nerve-fiber-layer changes after intravitreal injection of balanced salt solution.

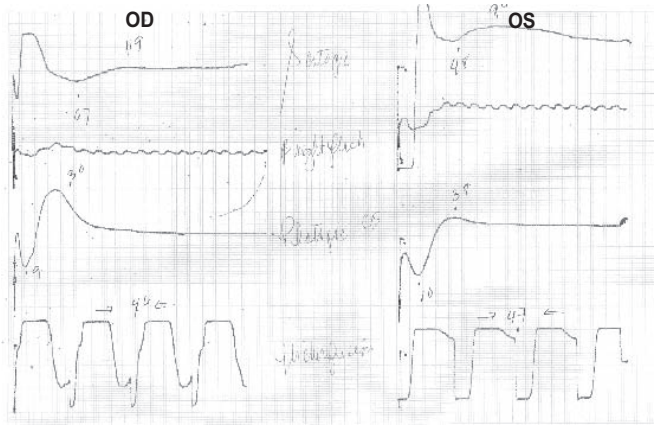


Figure 5. Electroretinography showing no significant variation between Group 1 (OD) and control group (OS).

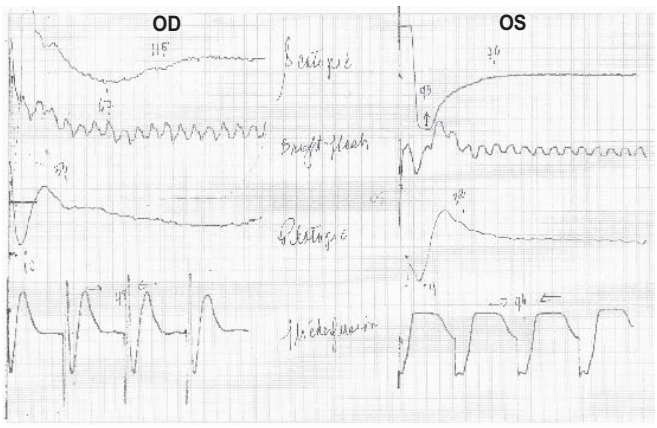
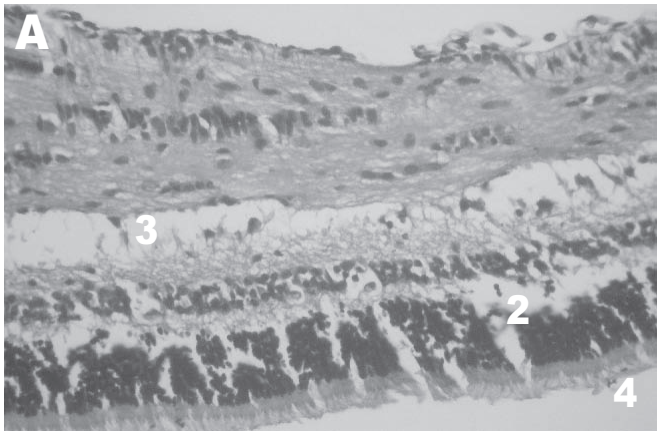
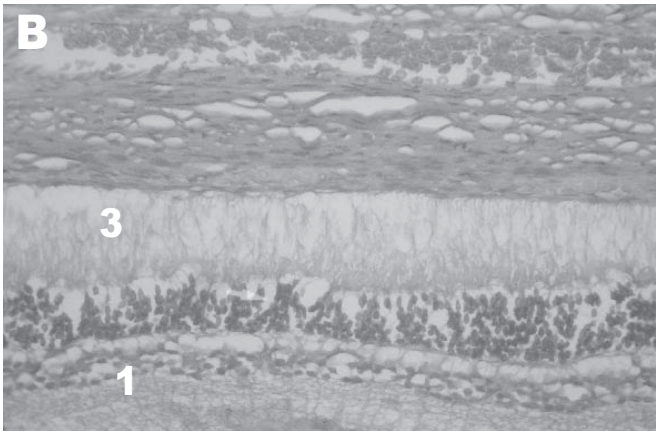


Figure 6. Electroretinography showing no significant variation between Group 2 (OD) and control group (OS).





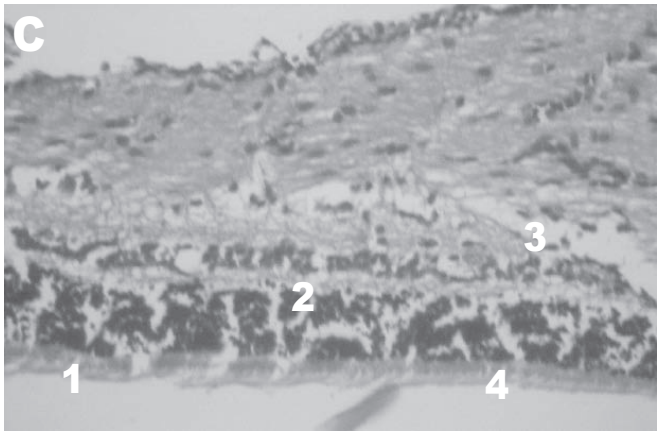


Figure 7. Histology of the rabbit retina in Group 1 [**A**], Group 2 [**B**], and control [**C**] showing inflammatory cells (**1**), vacuolization (**2**), decreased outer-nuclear-layer-cell density (**3**), and partial loss of photoreceptor outer segment (**4**).

No ERG implicit time and amplitude changes were observed in all the ERG tests on time-dependent comparisons. Except for a statistically significant decrease in the b-wave amplitude in the scotopic test of Group 2 without a concurrent implicit time change observed between 3 hours and 2 days and between 3 hours and 7 days after the administration of linezolid, there were no other implicit time or amplitude changes observed in the other ERG tests on dose-dependent comparisons. The decrease in b-wave amplitude was only noted at 3 hours and not after 2 days, implying a transient effect on the retina. Morever, the change was noted only in the scotopic test. Further investigations are needed to determine a true toxic effect of linezolid on the retina at higher doses.

Histopathologic examination showed minimal inflammatory changes and absence of necrosis in the retinal layers in all groups. Mild changes such as vacuolization, decreased cell density, and partial loss of photoreceptor outer segment were seen in both the treated and the control groups.

Histologic studies of the retina are important in evaluating the safety of a drug because the photoreceptor and other retinal cells directly adjacent to the vitreous are highly sensitive not only to the offending pathogen and the resulting inflammatory response but also to high doses of antimicrobial agents administered intravitreally to treat the infection.[1, 13-16]

Intravitreal antibiotic therapy has been the principal mode of treatment for acute bacterial endophthalmitis since this route results in a far greater intraocular antibiotic concentration than any other method of administration. Because of the importance of prompt treatment and the inaccuracies of gram-staining results, broad-spectrum intravitreal antibiotics effective against both gram-positive and -negative bacteria are usually administered even before the culture results are available.

Linezolid has demonstrated a wide spectrum of activity against different microorganisms, and its ability to kill resistant strains warrants its evaluation as a possible alternative to intravitreal vancomycin. Further studies, however, are needed to determine its effects in human eyes even though *in vitro* studies in rabbit eyes showed no toxic effects on the vitreous and the retina.

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