

Intravitreal antibiotic therapy with vancomycin and aminoglycoside: examination of the retinal toxicity of repetitive injections after vitreous and lens surgery *

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Abstract. The combination of vancomycin and an aminoglycoside antibiotic is frequently injected into aphakic/vitrectomized eyes for the treatment of endophthalmitis. At 2 weeks after lens and vitreous removal, rabbit eyes received an injection of a combination of 1 mg vancomycin and either 400 µg amikacin or 100 µg gentamicin. Furthermore, the effects of repeating the same combination a second and third time at intervals of 48 h were examined by light and transmission electron microscopy at 7 days to 4 months after the last injection. A single injection of either combination produced no toxicity. After 2 or 3 sets of injections of either combination, 13 of 16 eyes displayed retinal toxicity manifested by macrophages in the subretinal space, disorganization of the outer segments and retinal pigment epithelium, and discontinuities in Bruch's membrane. These results indicate that whereas a single injection of these combinations is not toxic to aphakic/vitrectomized eyes, repetitive injections may result in increasing toxicity.

Introduction

The rapid treatment of endophthalmitis requires antibiotic administration prior to identification of the causative organism [6, 14]. A combination of drugs is necessary to provide the broad-spectrum coverage required. For this reason, the combination of vancomycin and an aminoglycoside antibiotic is currently recommended for the initial intravitreal therapy of bacterial endophthalmitis [6, 10, 15, 19]. These drugs are commonly given at the conclusion of vitrectomy.

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Previous studies have documented that a single intravitreal administration of a combination of these agents is nontoxic to the retina in the nonsurgically treated rabbit eye [10]; however, these prior studies had important limitations. Specifically, the most typical patient exhibiting endophthalmitis after surgery has undergone lens removal and subsequent vitreous removal as part of the therapy. Lens and vitreous removal are known to accelerate the clearance of intravitreally injected substances [1, 2, 8, 12], and some authors have suggested that repetitive injections may be necessary in patients for the maintenance of adequate drug levels in the eye [2, 8, 12]. Alternatively, others have been concerned about possible increases in retinal toxicity in surgically altered eyes, suggesting a possible retina-protective effect of formed vitreous [3, 4, 11].

The present study examines the complex interrelationships between vitreous and lens surgery and repetitive treatment with combinations of intravitreal antibiotics. These operative conditions are important, as they more closely approximate the clinical setting in which human eyes are treated for bacterial endophthalmitis.

Materials and methods

A total of 40 Dutch Belted rabbits weighing 2-3 kg were used for these experiments. Animals were anesthetized with a 1.5-ml mixture of equal parts of xylazine (20 mg/ml) and ketamine hydrochloride (100 mg/ml) given in an intramuscular injection. The pupil was dilated with 1% tropicamide, 5% phenylephrine hydrochloride, and 1% atropine sulfate, and the surgical field was prepared with povidone-iodine soap.

Under the operating microscope, a fornix-based conjunctival flap was prepared. A myringotomy blade was used to create two sclerotomies 1 mm posterior to the limbus at the 10 and 2 o'clock positions. At the 10 o'clock position, the blade was advanced through the sclera into the lens nucleus. An infusion cannula attached to a bottle containing lactated Ringer's solution was inserted into the sclerotomy at the 2 o'clock position and secured with a mattress suture. A phakofragmenter was inserted into the eye and lensectomy was performed using ultrasound, with care being taken to preserve the anterior capsule. An irrigating contact

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Table 1. Experimental protocol

Drug combination	Times injected	Number of eyes
1 mg vancomycin and 400 µg amikacin:	1	5
	2	4
	3	4
1 mg vancomycin and 100 µg gentamicin:	1	5
	2	4
	3	4
Saline:	1	3
	2	3
	3	3
No injection (surgery only)	—	5

lens was then placed on the cornea and vitrectomy was performed using a vitreous cutter. Just before removal of the probe, the anterior lens capsule was incised and removed. The sclera and conjunctiva were closed with sutures. Eyes were treated with 1% atropine sulfate ointment once a day for 2 weeks after surgery before being used for evaluations of toxicity. At 2 weeks postsurgery, by which time the inflammation had subsided, the selected drugs were injected sequentially into the midvitreal cavity via a 30-gauge needle inserted through the limbus. Prior to intravitreal injections, an anterior-chamber paracentesis was performed.

The rabbits were randomly divided into four groups comprising three or more rabbits each (Table 1). The first group received 1 mg vancomycin and 400 µg amikacin, the second group was given 1 mg vancomycin and 100 µg gentamicin, the third group received sterile, preservative-free saline, and the fourth group, which received no injections, served as an additional control of the effects of surgery only. Antibiotic doses were adjusted such that the total volume of drug or saline was always 0.2 ml. Animals in the first three groups were randomly subgrouped to receive a second or third set of identical injections (Table 1). The second set of injections were given at 48 h after the first; similarly, the third set of injections were carried out at 48 h after the second set.

Animals were periodically examined by indirect ophthalmoscopy and were photographed after pupillary dilation. They were killed at intervals ranging from 7 days to 4 months after the last injection. The eyes were immediately enucleated, hemisected at the equator, and subsequently fixed in 2.5% glutaraldehyde for 24–48 h. After a buffer wash, the retina was dissected into 2-mm² fragments, postfixed in 1% osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon. Semithin sections were stained with methylene blue-azure II for examination by light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate and examined by transmission electron microscopy.

Results

Indirect ophthalmoscopy and inspection of calottes after surgery only or following a single injection of saline or antibiotics disclosed no fundus change. However, scattered areas of retinal folds developed in approximately one-half of the eyes after two intravitreal injections of both antibiotics or saline and in two-thirds of the eyes after three injections (Fig. 1). These retinal folds occurred at 2–3 days after the final injection and showed no change for as long as 4 months. Histological examination (Fig. 2) revealed that the neural retinal layers were elevated from the pigment epithelium and folded inwardly. Within an individual fold, photoreceptor outer

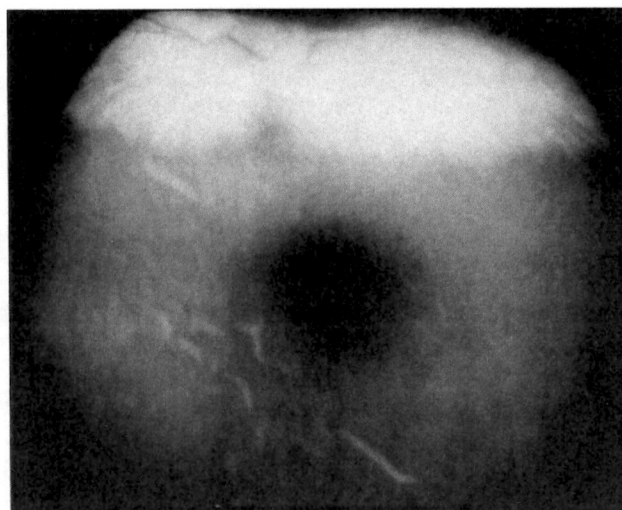


Fig. 1. Fundus photograph taken 5 days after three injections of vancomycin/gentamicin. Retinal folds are visible in the posterior retina

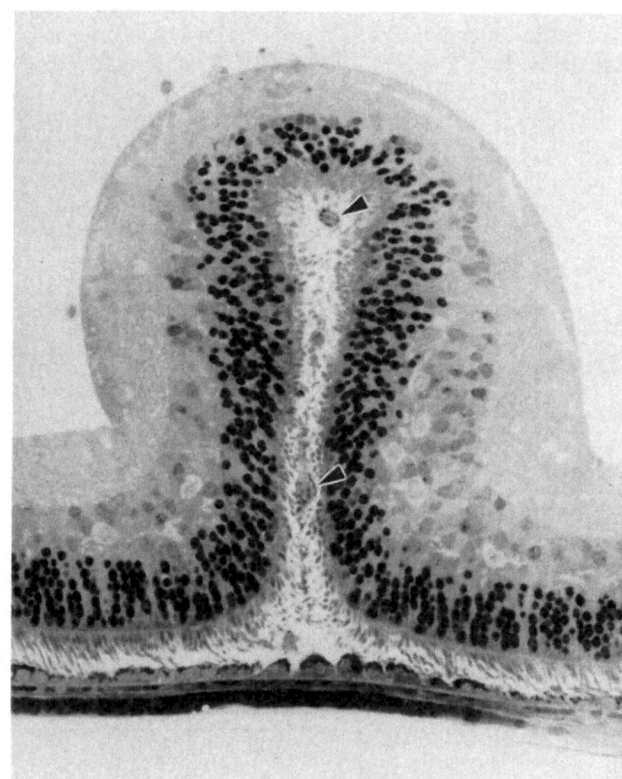


Fig. 2. Light micrograph obtained 1 week after three injections of vancomycin/gentamicin. The neural retina is elevated from the retinal pigment epithelium and folded inward. Macrophages (arrowheads) are visible within the area of degenerated photoreceptor outer segments. Azure II and methylene blue stain, $\times 250$

and inner segments facing each other were degenerated, compressed, and mixed with cellular debris. Macrophages containing lysosomes were often found in the subretinal space, and the underlying retinal pigment epithelium was usually tall, swollen, and retracted toward

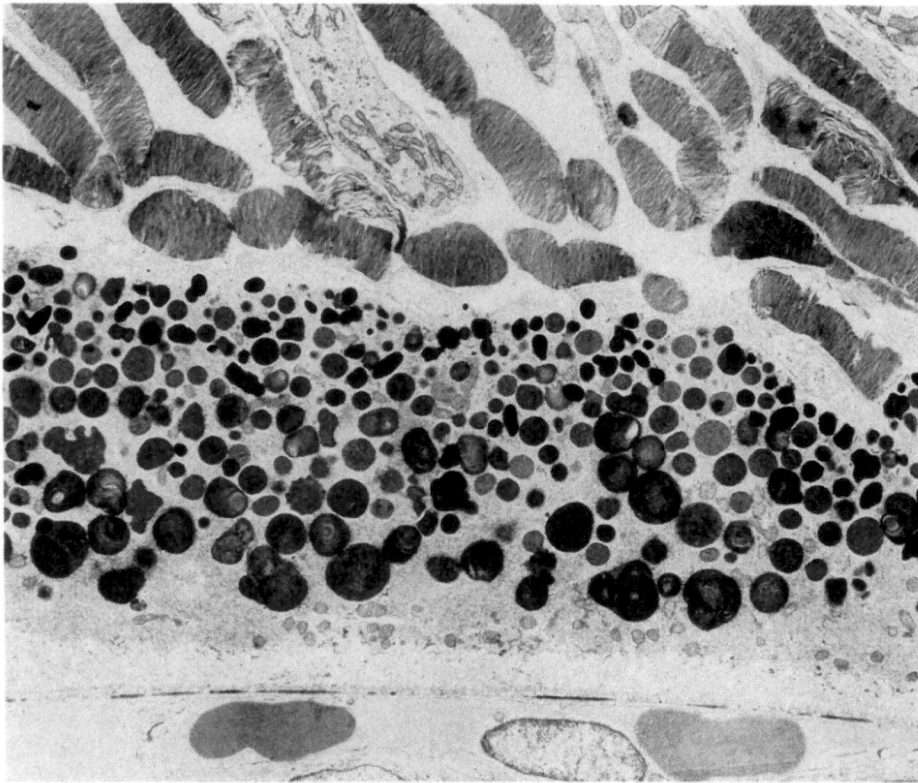


Fig. 3. Transmission electron micrograph obtained 1 week after two injections of vancomycin/amikacin, showing an accumulation of lysosomes in the retinal pigment epithelium. Uranyl acetate and lead citrate stain, $\times 2950$

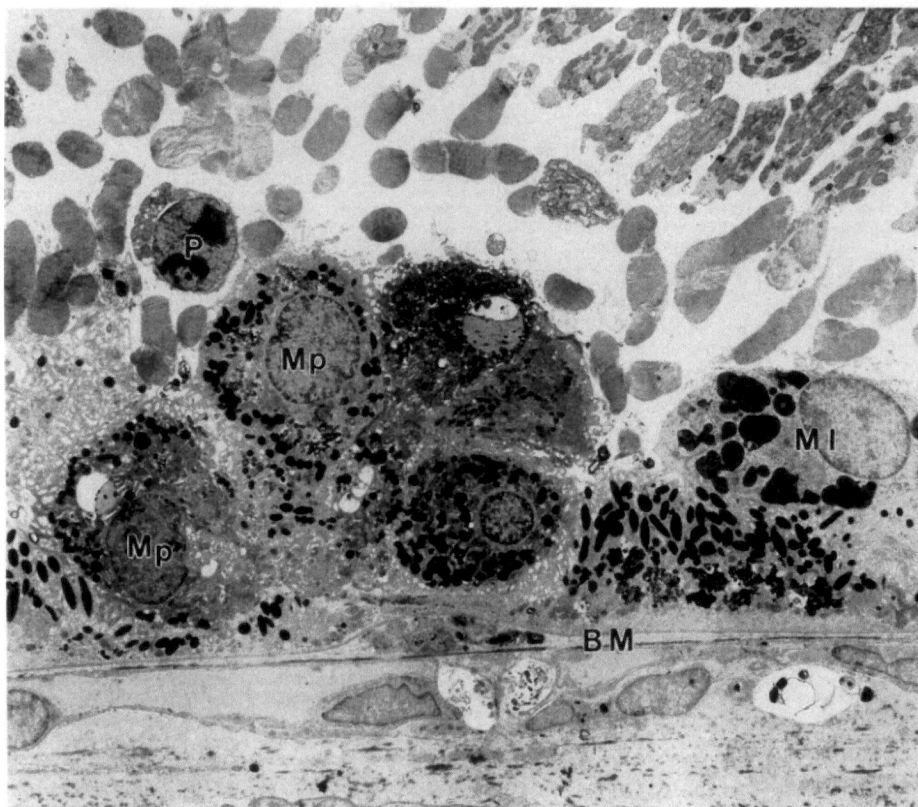


Fig. 4. Transmission electron micrograph obtained 4 months after three injections of vancomycin/amikacin. Macrophages containing pigment (*Mp*) that appears to have been derived from the retinal pigment epithelium, a macrophage containing nonpigmented lysosomal inclusions (*MI*), and a photoreceptor cell nucleus are visible in the subretinal space. *B-M*, Bruch's membrane. Uranyl acetate and lead citrate stain, $\times 1650$

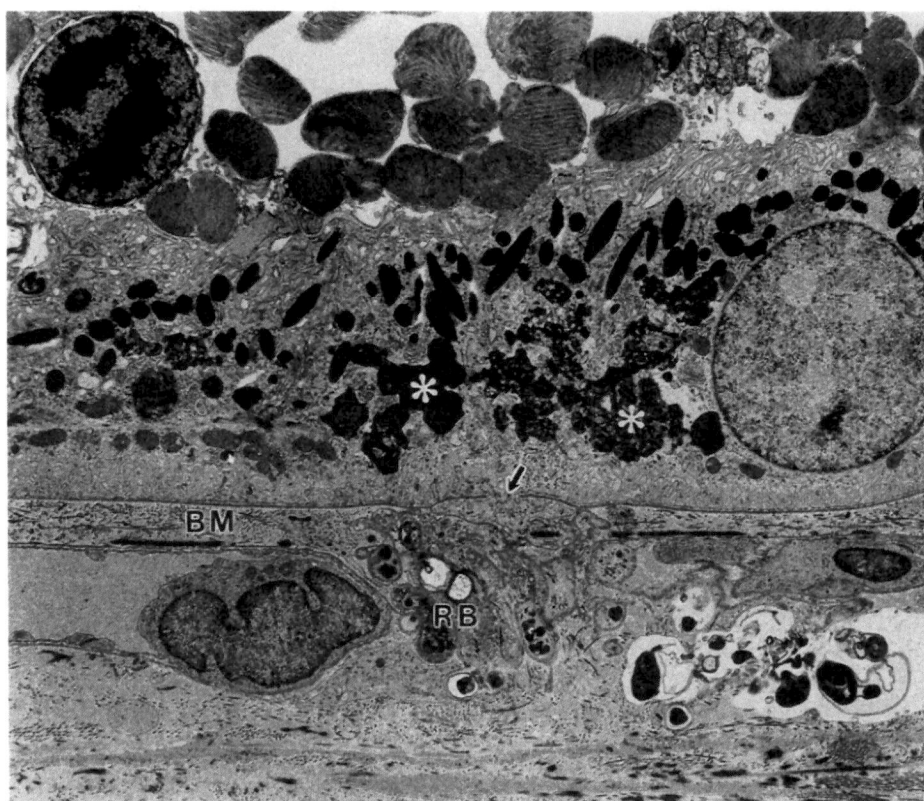


Fig. 5. Transmission electron micrograph obtained 4 months after three injections of vancomycin/amikacin. Lysosomal material appears to have coalesced and formed dense fibrillar or amorphous collections (asterisks) within the hypertrophied retinal pigment epithelium. Cell membranes of the retinal pigment epithelium appear discontinuous (arrow) in association with the discharge of residual bodies (RB) into the choroid. Uranyl acetate and lead citrate stain, $\times 3810$

the top of the retinal fold. The retina intervening between the folds was unremarkable.

Light microscopic and ultrastructural evaluation disclosed no evidence of retinal toxicity between control eyes and eyes receiving a single set of either combination therapy. Apart from the occasional retinal folds described above, control eyes receiving only surgery or surgery along with one to three saline injections displayed rare focal areas in which the retinal pigment epithelium was pleomorphic, although not to the degree observed in retinal toxicity. Inflammatory cells were observed within the vitreous along the internal limiting membrane in all eyes examined, and the number of these cells increased slightly with additional injections.

After two injections, two of four eyes treated with amikacin plus vancomycin and three of four eyes treated with gentamicin plus vancomycin displayed focal areas of retinal toxicity that exhibited similar pathologic features on histologic examination. After three sets of injections, all eight eyes receiving either combined therapy displayed histologic evidence of more advanced retinal toxicity. Typical pathologic features included hypertrophy and hyperplasia of the retinal pigment epithelium, the occurrence of numerous lamellar lysosomal inclusions in the retinal pigment epithelium (Fig. 3), the appearance of lysosome-containing macrophages in the subretinal space, and the disorganization and loss of photoreceptor outer segments associated with preservation of the inner segments. At 4 months, these findings persisted and the cellular inclusions coalesced to form

a large, dense, fibrillar mass in the retinal pigment epithelium and in subretinal macrophages (Fig. 4). In some areas, phagosomes in the photoreceptor outer segments appeared to penetrate through a break in the cytoplasmic membrane and basement membrane of the retinal pigment epithelium to enter the inner collagenous layer of Bruch's membrane and continued to penetrate Bruch's membrane completely, reaching the intercapillary zone of the choroid. In other areas, partial closure of the break in the cytoplasmic membrane of the retinal pigment epithelium was observed; this finding is consistent with the apparent passage of cellular debris into the choroid (Fig. 5).

Discussion

The present study documented that a single administration of a combination of vancomycin and aminoglycoside was well tolerated in the rabbit eye, even in the absence of the lens and vitreous. However, repetitive injections of the same combination of antibiotics resulted in increasing retinal toxicity. These results raise questions regarding the use of more than one set of intravitreal antibiotic injections in the subsequent management of patients with endophthalmitis, including the large subgroup that has undergone lens and vitreous removal.

Rapid treatment of bacterial endophthalmitis is essential and requires the administration of intraocular

antibiotics prior to the disclosure of culture results [6, 14]. As no single antibiotic available possesses the necessary broad-spectrum activity, a combination of antibiotics is required. The combination of vancomycin (for gram-positive coverage [12, 15] and an aminoglycoside (either amikacin or gentamicin, for gram-negative coverage) [10, 19] is currently recommended for the therapy of bacterial endophthalmitis. These antibiotics are frequently given at the conclusion of vitrectomy and are most commonly used in eyes that have recently undergone lens removal as well.

Repetitive intravitreal injections have been considered in the past on the basis of pharmacological considerations. Removal of the lens and vitreous has been demonstrated to accelerate the clearance of intravitreally injected substances [1]. Cobo and Forster [2] determined a half-life of 32 h for intravitreal gentamicin in nonsurgically treated rabbit eyes as compared with 12 h in aphakic eyes. These authors calculated that the optimal time for reinjection of intravitreal gentamicin would be 36–48 h in aphakic cases and 72–96 h in phakic cases for the maintenance of effective antibiotic levels in the eye. Pflugfelder and associates [12] found that a therapeutic level of intravitreally injected vancomycin was present for 72 h in phakic eyes and for 48 h in aphakic/vitrectomized eyes exhibiting an intact lens capsule; however, the additional removal of the lens capsule caused the vitreous concentration to fall to subtherapeutic levels by 48 h. Maurice [8] calculated a strategy in which intravitreal drugs would be reinjected at the time by which the remaining drug had declined to 10% of the initial dose. These observations have led to the idea of repetitive intravitreal antibiotic injections in eyes exhibiting endophthalmitis that have undergone lens or vitreous removal. Conversely, other investigators have been concerned that surgically altered eyes may be at greater risk of retinal injury, postulating a retina-protective effect of formed vitreous [3, 4, 11].

The results of the present study are in close agreement with the findings of a previous study on nonsurgically treated eyes [10] and suggest that lens and vitreous removal neither enhance nor decrease the safety of intravitreal combinations of vancomycin and aminoglycoside. In a study on the use of intravitreal aminoglycosides alone, Talamo and associates [18] found no difference in the single-dose toxic threshold in normal, aphakic, or aphakic/vitrectomized rabbit eyes, and they linked the retinal toxicity of the drug to the peak level occurring immediately after injection in the eye and not to its rate of disappearance. Recently, Tabatabay and associates [17] used immunocytochemical techniques to document the rapid movement of intravitreally injected gentamicin across the retina and into the retinal pigment epithelium. It remains concentrated there for ≥ 48 h, suggesting a mechanism for the outer retinal toxicity observed following excessive doses of gentamicin.

It appears that retinal toxicity due to repetitive intravitreal injections in patients has been documented by Olson and associates [9]. These authors noted retinal atrophic changes associated with pigment clumping at the level of the retinal pigment epithelium in two patients

following repeated intravitreal injections of combined gentamicin and cephalosporin for the treatment of endophthalmitis. Although these abnormalities appeared similar to those observed in experimental aminoglycoside toxicity, the authors could not determine whether these changes resulted from repetitive antibiotic injection or from the underlying bacterial infection.

The development of retinal folds in animals has been well documented to occur spontaneously and to be attributable to a wide variety of factors including surgery, intravitreal injection, developmental anomalies, and radiation [7, 13, 20]. Because retinal folds occurred in our experiments in eyes receiving surgery and multiple injections of saline or antibiotics, we attributed their development to physiologic consequences of surgery as well as repeated paracentesis and injections and did not consider them to be a pharmacologic manifestation of drug toxicity. Poulson and Hayes [13] reported congenital retinal folding in rats, the majority of which had been given intravitreal injections; in their report, however, these authors discounted the possibility that the intravitreal injection might have produced the folds.

The specific features of the retinal toxicity caused by intravitreal injection of vancomycin and aminoglycoside antibiotics in the rabbit have previously been characterized following individual as well as combination drug therapy and are most evident in the outer retina and retinal pigment epithelium [5, 10, 12, 15]. The retinal abnormalities observed in the present study in aphakic/vitrectomized eyes following two or three sets of injections of a combination of vancomycin and aminoglycoside antibiotics are similar to those previously reported and include the appearance of macrophages in the subretinal space, the occurrence of numerous lamellar lysosomal inclusions in the retinal pigment epithelium, the development of focal areas of retinal pigment epithelial hypertrophy and hyperplasia associated with clustering of pigment granules in the apical cytoplasm of the retinal pigment epithelium, and the disorganization of photoreceptor outer segments. These findings persisted throughout the 4 months of the study, as did further collection of cellular debris in the retinal pigment epithelium, which possibly extended across Bruch's membrane into the choroid. The histologic techniques used in this study suggest that a drug-induced outer retinal and retinal pigment epithelial abnormality occurred, resulting in basally directed movement of outer retinal debris across discontinuities in Bruch's membrane and into the choroid. A previous study on intravitreal aminoglycoside in normal rabbit eyes has also documented the accumulation of drusen-like lesions several months after a single drug treatment [16].

These experimental results have implications for the care of patients presenting with endophthalmitis. The findings suggest that patients should receive only one intravitreal injection of the combination of vancomycin and aminoglycoside, and this guideline would also apply to the large subgroup of patients who have undergone lens and vitreous surgery as well. For purely pharmacologic considerations, repetitive intravitreal injections would not be recommended for the great majority of

cases, regardless of the observation that vitreous drug clearance is known to be accelerated in surgically altered eyes. Repetitive injections would be reserved for the small subgroup exhibiting a persistent active infection following the initial vitrectomy and injection of intravitreal antibiotics, in whom the risk of cumulative retinal toxicity is outweighed by the devastating consequences of uncontrolled intraocular infection. Further study of the roles of vitrectomy and intravitreal antibiotic therapy and their interrelationship will continue to improve the prognosis of patients presenting with bacterial endophthalmitis.

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