Acute Aminoglycoside Retinal Toxicity In Vivo and In Vitro

Heather A. Hancock,¹,² Clyde Guidry,³ Russell W. Read,³,⁴ Edgar L. Ready,⁵ and Timothy W. Kraft⁵

PURPOSE. Intentional and inadvertent intraocular administration of aminoglycosides is associated with cases of retinal toxicity. Clinical manifestations resemble a vaso-occlusive event and include edema, intraretinal hemorrhage, and nonperfusion detected by fluorescein angiogram. This study was conducted to measure the retinal function in avascular and isolated perfused retinas to separate vascular and neurologic effects of gentamicin. Enhanced understanding of the mechanism of gentamicin toxicity may lead to development of aminoglycosides that can be used to treat ophthalmic infections without retinal damage.

METHODS. Whole animals and isolated rabbit and rat retina preparations were used to study the dose dependence and reversibility of toxicity on the ERG, with a 1- and a 10-mg/mL solution of gentamicin. The amplitude and implicit times of the a-, b-, and c-waves were measured before, during, and after exposure to the drug.

RESULTS. In whole-animal ERG studies, intraocular administration of gentamicin eliminated the b-wave but left the a-wave intact. The c-wave was reduced in amplitude. Histopathologic evaluation demonstrated diffuse disruption of the nerve fiber layer and the inner plexiform layers. Isolated retinal studies showed that the b-wave was reduced in amplitude in the presence of low-dose gentamicin (1 mg/mL) and completely eliminated by high-dose gentamicin (10 mg/mL). This effect was reversible for short-term exposure to gentamicin.

CONCLUSIONS. The results indicate that the initial loss of function due to exposure to aminoglycoside antibiotics is independent of the vascular supply. Elimination of the b-wave was dose dependent and reversible, indicating that a component of gentamicin toxicity is mediated through pathways other than vascular supply. Short-term effects are reversible, suggesting a receptor-mediated process. (Invest Ophthalmol Vis Sci. 2005;46:4804 – 4808) DOI:10.1167/iovs.05-0604

The aminoglycosides are a class of drugs with antibiotic action secondary to their ability to inhibit bacterial protein synthesis at the ribosome.¹ Commonly used aminoglycosides include tobramycin, amikacin, and gentamicin. Gentamicin has been used in the treatment of endophthalmitis and anterior segment infection and for postoperative prophylaxis because of its activity against Gram-negative organisms.² It is cleared from the eye by the anterior chamber and has a half-life of 33 hours.³ Although aminoglycosides are well known to be associated with oto-⁴ and nephrotoxicity⁵ with systemic use, it has recently been recognized that there is ocular toxicity with local administration as well.

Several cases of retinal toxicity have been reported after aminoglycoside use for the treatment or prophylaxis of endophthalmitis. Loss of visual function has been reported after administration of gentamicin, amikacin, and tobramycin.⁶ Acutely, the retina becomes opaque and edematous with superficial and intraretinal hemorrhages, cotton wool spots, arteriolar narrowing, and venous beading. Fluorescein angiography reveals severe vascular nonperfusion,⁷ and ERG responses are extinguished. Chronic findings include neovascular glaucoma, pigmentary degeneration, optic atrophy, and severe visual loss.⁸ After inadvertent intraocular injection of aminoglycoside, vision loss can, in some cases, be prevented with lavage of the anterior chamber and early vitrectomy.⁹ Several studies of aminoglycoside toxicity in animal models have shown vascular changes similar to those in clinical cases. A 10-mg injection of gentamicin in primate vitreous caused whitening of the retina, retinal hemorrhages, multiple areas of vascular leakage, and loss of the a- and b-wave from the ERG. These changes mimic acute central retinal artery obstruction.¹⁰ Conway et al.¹¹ demonstrated marked swelling of the nerve fiber layer and ganglion cell necrosis with preservation of normal vasculature after intravitreal injection of 1 mg gentamicin. Hines et al.¹² showed that vacuolization of the nerve fiber layer and disruption of the inner nuclear layer occur within 12 hours of gentamicin administration, followed later by vascular congestion and leukocyte margination. Mochizuki et al.¹³ described loss of the a- and b-waves and the oscillatory potentials 7 to 14 days after a 0.24-mg intravitreal injection of gentamicin in rabbits.

The mechanism of retinal toxicity of aminoglycosides remains unclear. The striking clinical presentation of capillary nonperfusion after aminoglycoside administration has suggested a primarily vascular mechanism. Because gentamicin is a strong acid, it has been suggested that toxicity may be a direct pH effect¹⁴ or that it may accumulate in the acidic environment of the lysosome and cause disruption of RPE metabolism.¹⁵ To elucidate further the mechanism of the retinal toxicity of aminoglycosides, we examined both whole-animal ERGs in rabbits and the in vitro ERG responses of rabbit and rat retinas, both of which represent retinal preparations removed from their normal blood supply. The isolated retinal preparations also facilitated rapid and reversible exposure of the retina to gentamicin. Elucidation of the mechanism of gentamicin toxicity may lead to development of aminoglycosides that can be used to treat ophthalmic infections without retinal damage.

METHODS

Isolated Retinal Preparation

Animals were handled according to the principles of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.
isolated retinal preparations (n = 5), animals were dark adapted for at least 12 hours before death, rats were killed by carbon dioxide asphyxiation and rabbits by pentobarbital sodium overdose. Eyes were enucleated with IR-sensitive goggles (AN/PVS-5 Nightvision Goggles; IIT, Roanoke, VA [among others]), eye cups were prepared (Leibovitz L-15 medium; Invitrogen-Gibco Corp., Carlsbad, CA), and neural retinas were teased away from the RPE. All subsequent preparation was performed in the dark with the aid of infrared image converters (Dark Invader; BE Meyers Inc., Redmond, WA) attached to the dissecting microscope eyepieces.

A disc of retina with approximately 6-mm diameter was mounted in a Ussing chamber (World Precision Instruments, Sarasota, FL) modified to receive a fiber optic. The photoreceptor side of the retina faced the fiber optic, whereas the ganglion cell layer (GCL) side was supported by a filter paper. Electrodes connected to the Ussing chamber consisted of Ag/AgCl pellets. The Ussing chamber was attached to a system that perfused warm, bubbled (95% O₂:5% CO₂) Ames’ medium at a rate of approximately 1 mL/min. Flow was calibrated at the beginning and end of each experiment. Two perfusion lines delivered Ames medium from the heated beaters to the Ussing chamber. One line delivered control medium to the photoreceptor side, and a second line fed the ganglion cell side. This line was configured to deliver either control medium or medium containing gentamicin. The Ussing chamber was perfused with warm solutions heated in water-jacketed beaters (Radiant, Monrovia, CA) and oxygenated through plastic tubing. The water bath was heated and circulated with a temperature-controlled water pump (Polyscience, Niles, IL). Perfusion lines were heated with a DC power supply (Topward; SpenceTek Inc., Milpitas, CA). The temperature was calibrated at the location of the retina to 37°C. After baseline isotropic ERG responses were recorded (DC to 300 Hz), test medium containing 1 or 10 mg/mL gentamicin was perfused onto the GCL side of the tissue and the responses recorded. Subsequently, the perfusate was switched back to control solution, and washout responses were recorded.

The light source was a 100-W tungsten-halogen lamp focused onto one end of a fiber optic. Stimulus duration was controlled with a shutter with a 6-mm aperture (Uniblitz, Vincent Associates, Rochester, NY). The energy output of the flashes was calibrated daily as the photon flux at the retinal surface in the Ussing chamber. Stimulus strength was controlled by a set of calibrated inconel neutral-density filters that allowed attenuation in steps of approximately 0.3 log units up to a maximum of 6.9 log units attenuation. The unattenuated stimulus was calibrated daily with an optical power meter (Graseby Optronics, Orlando, FL). We used a 505 nm (35 nm bandwidth) stimulus that was determined by a three-cavity interference filter (Anover Co., Salem, NH). For the isolated-tissue experiments the number of photoisomerizations (R*) is given by the product of the stimulus strength, I (photons per square micrometer at λmax), and Aα, the effective collecting area of the outer segment, calculated using the equation by Baylor et al.¹⁶ and Zhang et al.¹⁷:

\[ A_{\alpha} = V_{0\alpha} Q_{0\alpha} f / 2 \cdot 305a \]

where \( V_{0\alpha} \) is the volume of the outer segment, \( Q_{0\alpha} \) is the quantum efficiency of photoisomerization (0.67),¹⁸ \( f \) is a factor allowing for the use of unpolarized light entering the outer segment perpendicular to its long axis (\( f = 1 \) for end-on stimulus), and \( \alpha \) is the specific pigment density (0.016 μm−1).¹⁹ Based on volume measures from single-cell recordings, rat rods have an \( A_{\alpha} \) of 0.492 (Niculescu D, Kraft TW, personal communication, November 2004). The volume of the rabbit rod outer segments was calculated as 27.2 μm³ using the length of 15.4 μm reported by Tucker et al.²⁰ and assuming a diameter of 1.5 μm, giving an \( A_{\alpha} \) of 0.672 for rabbit rods.

**Corneal ERGs**

Rabbits (n = 2) were first anesthetized with xylazine (10 mg/kg intramuscularly) and ketamine (50 mg/kg intramuscularly) and intubated for administration of a constant dose of 3% isoflurane (Attane; Minrad, Inc., Bethlehem, PA). Rabbits were kept warm by a heating pad (Braintree Scientific, Braintree, MA) during recording. They were dark adapted for at least 45 minutes before recording. Corneas were anesthetized with 0.5% proparacaine (Bausch Lomb, Tampa, FL) and the pupils dilated with topical 2.5% phenylephrine HCl (Bausch Lomb) and 1% tropicamide (Alcon Laboratories, Inc., Fort Worth, TX). In addition, a solution of 2.5% methylcellulose (Goniosol; CIBA Vision Corp., Duluth, GA) was used during and after the recording session to keep the corneas moist until the animal recovered from anesthesia. Gentamicin was administered by vitreous tap after baseline data collection. A 27-gauge needle was used to remove 0.1 mL of liquefied vitreous from 3.0 mm posterior to the limbus in the superotemporal quadrant of the eye. A 0.1 mL aliquot of 100 mg/mL gentamicin and buffered saline solution was then injected into the vitreous cavity of one eye of each animal and 0.1 mL buffered saline (pH 7.0) was injected into the contralateral eye with the identical technique.

An electrode designed after that described by Lyubarsky and Pugh²² was placed on both eyes. Because of the large diameter of the electrode contacting the cornea (0.8 cm), an internal radius was added to act as a diverging lens.²² A platinum wire loop was affixed to the tapered end of a Plexiglas rod that had been hollowed out to receive the fiber optic that delivered the light stimulus. This design ensured a constant distance between the fiber optic and the eye and also acted as a diffusing element. A second identical setup was placed on the other eye and served as the neutral electrode. To record the ERG from the control eye, we simply removed the fiber optic from one electrode holder and placed it in the other. Responses were amplified 5000× under DC conditions with an amplifier (Astro-med CP122W; Grass Telefactor, W. Warwick, RI). The ERG voltage and stimulus monitor signals were digitized with hardware (MO16) and software (LabView) from National Instruments (Austin, TX). Data were sampled at either 0.5 or 1.0 ms/point, and the ERG signal was low pass filtered at 500 Hz. A stimulus set typically consisted of 3 to 20 responses to the same intensity of light repeated at 2- to 10-second intervals. The energy output of the flashes was calibrated daily as the photon flux emitted from the fiber optic.

**Histology**

After 2 hours of ERG recording, both eyes were enucleated, the tissue fixed in 10% neutral-buffered formalin and embedded in paraffin. Serial sections through each globe were obtained and every fifth section stained with either toluidine blue or hematoxylin and eosin. Sections were examined by light microscopy by a single ophthalmic pathologist (RWR) in a masked fashion.

**RESULTS**

To examine the effect of intracocular gentamicin on retinal physiology, we performed ERGs on anesthetized rabbits. After baseline recording, scotopic conditions were maintained while a solution of 10 mg gentamicin was injected into the test eye, and a sham solution of buffered saline at the same pH was injected into the contralateral eye. DC ERGs performed before injection showed typical a- and b-waves. The response to a 4-second step of light revealed the c-waves (Fig. 1, black traces). Injection of buffered saline did not affect the ERG (Fig. 1a, gray trace), whereas an injection of a solution of gentamicin eliminated the b-wave but left the a-wave intact (Fig. 1b, gray trace). The c-wave was reduced in amplitude. The inset in Figure 1 shows the b-wave on an expanded time axis. A similar result was obtained when this experiment was repeated in a second animal. After 2 hours of recording, the animal was killed and the eyes preserved for histologic examination. Artifactual retinal detachment was present in some areas of both saline- and gentamicin-injected eyes. Histopathology of retinas from animals injected with gentamicin solution re-
revealed a greater degree of vacuolization of the nerve fiber layer and edema of the inner plexiform layer of the gentamicin-injected eye than in the saline-injected eye (Fig. 2). All histologic sections underwent identical, simultaneously applied fixation, embedding, and staining protocols. Despite this, sections of the saline-injected eye appeared more intensely stained, most likely a result of edema in the gentamicin-injected eye, with the increased fluid preventing dye uptake.

Isolated-tissue ERG experiments were performed to test the reversibility of the gentamicin effect and to test its effect in both avascular (rabbit; Fig. 3) and vascular (rat; Fig. 4) retinas. Figure 3a shows the dim flash response (0.2 R*/rod). A 16-μV response (black trace) was reduced to 6 μV in the presence of 10 mg/mL gentamicin (blue trace). Figure 3b shows that a 57-fold brighter flash elicited a 46-μV response (thick black trace), which was reduced to 30 μV in the presence of gentamicin (gray trace). The response recovered rapidly on washout of the drug (thin gray trace). A bright flash (Fig. 3c; 2260 R*/rod) produced a 100-μV a-wave and a small b-wave. The Müller cell contribution to the PIII response, also known as the slow-PIII, is very prominent, peaking at approximately 0.7 seconds and recovering with a time constant of approximately 1.1 seconds. Gentamicin eliminated the b-wave, but had no effect on the a-wave or the slow PIII (Fig 3c; inset, gray trace).

Figure 4 demonstrates rapid and complete reversibility of gentamicin's effect on the isolated rat tissue ERG. In addition, to investigate the dose-dependence of gentamicin's effect on the b-wave, we exposed the isolated rat retina to one of two concentrations of gentamicin solutions. Figure 4a shows that a dim-light flash (2.0 R*/rod) generated a modest a-wave and a b-wave of approximately 150 μV, which was reduced to less than half of its magnitude by exposure to gentamicin at a concentration of 1 mg/mL for less than 5 minutes. The thin gray trace in Figure 4a shows that the effect was completely reversible. Stimuli of 30 R*/rod produced the responses shown in Figure 4b. The b-wave produced by this bright flash was dramatically but reversibly reduced by a low concentration of gentamicin. A higher concentration of gentamicin (10 mg/mL) almost completely eliminated the b-wave response to a dim

**FIGURE 1.** Effect of injected gentamicin on the whole-animal ERG. ERGs were recorded in an anesthetized rabbit before and after intravitreal injection. Black traces: baseline ERG; gray traces: the ERG after injection of a control solution. (a) The control ERG had an amplitude and time course similar to the baseline ERG. The stimulus was a 4-second step of light (7.25 log photons/μm² per second), illustrated by the horizontal bar at the bottom of each panel. (b) The contralateral eye was injected with 0.1 mL 100 mg/mL gentamicin (pH 7.0). Insets: b-wave on an extended time axis.

**FIGURE 2.** Retinal edema after treatment with gentamicin. Left: H&E-stained control tissue. Right: H&E-stained gentamicin-exposed rabbit retinal tissue. Histopathology of retinas from animals injected with gentamicin solution revealed a diffuse vacuolization of the nerve fiber, ganglion cell, and inner plexiform layers compared with saline injected eyes. Less intense staining in the gentamicin-exposed tissue (right) was probably a result of the retinal edema. Scale bar, 50 μm.

**FIGURE 3.** Isolated rabbit retina ERG. Gentamicin reduced the b-wave but did not affect the a-wave or the slow PIII (Müller cell contribution). (a) The response to a dim flash (0.2 R*/rod) was reduced in the presence of gentamicin. (b) The b-wave was reduced in the presence of gentamicin but recovered after washout with a 57-fold brighter flash (114 R*/rod). (c) Responses to a bright flash (2260 R*/rod); gentamicin did not alter the a-wave or slow PIII (c, inset). Expansion of a- and b-waves on the time axis.
flash (Fig. 4c). This larger effect was also reversible (Fig. 4c; thin gray trace). The brighter flashes produced larger a-waves that were enhanced or unmasked by gentamicin. The b-wave was completely eliminated by the 10-mg/mL concentration of gentamicin (Fig. 4d); the a-wave (fast PIII) and the slower Müller cell component (slow PIII) remained. The effects of the high concentration of gentamicin were also completely reversible (thin gray traces).

DISCUSSION

Although the clinical picture of retinal blanching in cases of gentamicin toxicity suggests a vascular process, our results indicate that another mechanism exists that directly interferes with retinal function. We found similar changes in retinal function in the presence of gentamicin in the rat retina, which has both choroidal and retinal vascular beds, and in the rabbit retina, which has only choroidal circulation. Selective loss of the b-wave occurred both in the whole-animal ERG and in isolated tissue. Therefore, gentamicin-induced changes occurred independent of retinal perfusion. The fact that our results show rapid and reversible effects in isolated tissue suggests that the immediate loss of vision occurs via a neural mechanism. There are several possibilities that explain why an initially neural process leads to a clinically vascular picture. It was somewhat surprising that the b-wave of the ERG was selectively affected. The simplest explanation for this finding is that bipolar function is impaired by gentamicin. Another possible explanation is that Müller cell function is damaged by gentamicin, which impairs bipolar cell function. We have found that the presence of antibiotics, especially gentamicin, in culture media dramatically reduces the viability of freshly isolated Müller cells (Ready RL, et al. IOVS 2004;45:ARVO E-Abstract 721). It is well known that Müller cells have numerous functions including regulation of retinal potassium homeostasis, lactate formation, and glutamate uptake. If Müller cells are injured by gentamicin and subsequently unable to maintain electrolyte concentrations, then abnormal electrolyte concentrations may be reflected in the ERG. However, the c-wave of the ERG, which is in part due to a Müller cell potassium pumping function, was unchanged in our whole-animal experiments. Also, the slow PIII, which directly reflects the Müller cell function, was unchanged in our isolated-retina experiments. These findings argue against the idea that Müller cell toxicity is the origin of the ERG changes we observed.

The selective reduction in the b-wave in the presence of gentamicin could be explained by disruption of glutamate transport. Although neurons express glutamate transporters, glial cells are responsible for most uptake at the synapse. Impairment of this important function by the glial cells would increase concentration of glutamate in the retina. The light response generated by bipolar cells is a response to decreased glutamate in the outer plexiform layer. If glutamate uptake mechanisms were impaired, high concentrations of glutamate...
would blind bipolar cells to the light response of photoreceptors, causing the b-wave to be diminished but allowing the a- and c-waves to be maintained. Experiments that introduced glutamate analogues show reduced b-waves with delayed implicit times and unmasked a-waves. In our results as well, the a-wave was unmasked in the presence of gentamicin (Fig. 4), suggesting no loss of photoreceptor function in the presence of gentamicin’s effect that we observed in short-term exposures over long exposure to aminoglycosides and the reversibility of genetic changes after intravitreous injection of glutamate has been shown to induce retinal damage in rats. This explanation is consistent with both the irreversible changes caused by glutamate to retinal tissue in vitro leads to histologic damage. Furthermore, intravitreal injection of glutamate has been shown to induce retinal damage in rats. Our results clearly demonstrate a neurotoxicity of gentamicin that may or may not be linked to the vascular events already described. Further elucidation of the mechanisms of aminoglycoside toxicity may allow for the design of drugs free of the neurotoxic side effects.

References