Physiological and Toxicological Effects of Cefuroxime on the Albino Rabbit Retina

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PURPOSE. Intracameral cefuroxime was found to lower the risk of endophthalmitis after cataract surgery. The purpose of this study was to evaluate the retinal toxicity of cefuroxime in a rabbit model.

METHODS. Twenty-two albino rabbits were divided into two cefuroxime groups: low-dose (1mg/0.1 mL, n = 9) and high dose (10 mg/0.1 mL, n = 13). The right eye of each rabbit was injected with 0.1 mL cefuroxime solution (experimental eye) and the left eye with 0.1 mL saline (control eye). Electroretinogram (ERG) responses were recorded at 3 hours, 4 days, and 1, 2, and 4 weeks after injection. After 4 weeks, the rabbits were euthanized, the eyes were enucleated, and the retinas were prepared for histologic evaluation and GFAP immunostaining.

RESULTS. No functional (ERG) or histologic damage was found in rabbits in the low-dose group. In the high-dose group, a significant decrease in the ERG amplitudes of the experimental eyes was seen 3 hours after injection, followed by partial recovery during 4 weeks of follow-up. Retinal histology of experimental eyes revealed marked damage. GFAP immunoreactivity in Müller cells was expressed in rabbits belonging to both groups, although it was more extensive in the high-dose group.

CONCLUSIONS. ERG and histologic findings indicated that a dose of 1 mg cefuroxime, administered intravitreally, was not toxic to the rabbit retina. A dose of 10 mg, injected intravitreally, induced transient physiological effects, and was toxic to the rabbit retina, as was evident by the permanent reduction in the ERG responses and by the structural damage to the retina with signs of glial activation. (Invest Ophthalmol Vis Sci. 2011;52: 906–914) DOI:10.1167/iovs.11-8053

Cefuroxime (Zinacef; GlaxoSmithKline, London, UK) is a second-generation cephalosporin and a member of the β-lactam family of antibiotics. Administered by subconjunctival/intracameral injection, cefuroxime is frequently used as a prophylaxis for postoperative bacterial endophthalmitis in cataract surgery1,2 because it inhibits relevant bacterial strains and is associated with a low frequency of postoperative endophthalmitis.3

In 2007 the European Society of Cataract and Refractive Surgery (ESCRS) has published the results of a large prospective randomized multicenter study that was designed to assess the effect of antibiotic prophylaxis on the incidence of postoperative bacterial endophthalmitis after cataract surgery.4 Intracameral cefuroxime injection (1 mg/0.1 mL) during cataract surgery was found to be beneficial in reducing the risk of postoperative endophthalmitis. When no intracameral cefuroxime was used, the risk for endophthalmitis increased five- to sixfold.4 In light of these findings, an intraoperative, intracameral cefuroxime injection has been used routinely by many surgeons during cataract surgery.5

Cefuroxime may reach the vitreous more easily after intraoperative administration, in pseudophakic eyes, or when surgical complications such as posterior capsular tear and vitreous loss occur. Reports on cases in which a high dose of cefuroxime was inadvertently injected intracameral indicated transient retinal complications and raised the need to test the effects of the drug on the retina.6–8 Several previous animal studies have shown retinal toxicity of intravitreally injected cephalosporins9–14; however, only one study examined the potential retinal toxicity of cefuroxime.15 This study included ERG follow-up of four rabbits: two with a low dose (0.1 mg/0.1 mL) and two with a high dose (1 mg/0.1 mL). Neither dose was found to be toxic; however, the small number of animals tested and the large variability between them did not provide sufficient information on possible toxicity. We tested the effects of cefuroxime on the retina of albino rabbits at two doses: the one used routinely in cataract surgery (1 mg/0.1 mL) and a 10-fold higher dose (10 mg/0.1 mL).

METHODS

Animals

Twenty-two adult New Zealand White rabbits weighing 2 to 3 kg each were included in the study. The rabbits were housed in a 12/12-hour light-dark cycle and were allowed free access to water and food. Before intravitreal injection and electrophysiological recordings, the rabbits were anesthetized by an intramuscular injection of a mixture containing ketamine hydrochloride (10 mg/mL), acepromazine maleate solution (10%), and xylazine solution (2%), at a ratio of 1:0.2:0.3. Topical anesthesia (benoxinate HCl 0.4%) was administered to the eyes to reduce the animal’s discomfort. The pupils were fully dilated with cyclopentolate hydrochloride 1%. At the end of the follow-up period, the rabbits were euthanized by intravenous injection of an overdose of pentobarbital sodium (80 mg/kg body weight), and the retinas were prepared for histologic and immunocytochemical observations.

The rabbits were divided into two groups. The animals in the low-dose group (n = 9) were treated with intravitreal cefuroxime at a concentration of 1 mg/0.1 mL, as is used in clinical practice during
cataract surgery. Animals in the high-dose group (n = 13) were treated with cefuroxime at a concentration of 10 mg/0.1 mL.

All the experimental procedures adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and to institutional guidelines.

**Intravitreal Injection**

Intravitreal injections were performed as described previously. A 28-gauge needle attached to a 1.0-ml tuberculin syringe was inserted into the vitreous approximately 3 mm posterior to the limbus. The needle was directed to the middle of the globe, toward the region of the optic disc, and then a volume of 0.1 mL was slowly injected. The right eye was always injected with cefuroxime solution and is referred to as the experimental eye, and the left eye was injected intravitreally with 0.1 mL normal saline and served as control.

**Cefuroxime**

Cefuroxime sodium powder was dissolved in double-distilled water for injection, as instructed by the manufacturer. Two doses were used: 1 mg/0.1 mL (low dose) and 10 mg/0.1 mL (high dose). The high-dose solution osmolarity was 364 mOsmol, and its pH was 8.2. The low-dose solution osmolarity was 37 mOsmol, and its pH was 8.02.

**Clinical Observation**

Each rabbit underwent clinical inspection with an indirect ophthalmoscope after the injection and at every time point during the follow-up period, for signs of ocular inflammation, cataract formation, and retinal damage.

**Electroretinogram**

Flash ERG responses were recorded simultaneously from the experimental and control eyes using corneal electrodes (Medical Workshop, Groningen, The Netherlands). The reference and ground electrodes, made of stainless steel surgical needles, were inserted into the ears. The ERG signals were amplified (×20,000) and filtered (0.3–300 Hz) by differential amplifiers (Grass Telefactor, West Warwick, RI). Light stimuli were obtained from a Ganzfeld light source (LKC Technologies, Gaithersburg, MD) with a maximum energy of 5.76 cd·s/m².

The ERG responses were recorded in the dark-adapted state (at least 30 minutes in darkness), for assessment of rod system function, and then in the light-adapted state (background illumination of 1.15 cd/m²), for cone system evaluation. Six responses elicited by identical flash stimuli at 10-second intervals were averaged in the dark-adapted state, and 15 responses elicited at a frequency of 1 Hz were averaged in the light-adapted state.

ERG analysis was based on amplitude measurements of the a- and b-wave. The a-wave amplitude was measured from the prestimulus baseline to the trough of the a-wave, and the b-wave was measured from the trough of the a-wave to the peak of the b-wave. The b-wave amplitudes of the experimental and control eyes were plotted as a function of log stimulus energy and fitted to a generalized Michaelis-Menten-type hyperbolic function:

\[
\frac{V}{V_{\text{max}}} = \frac{P}{P + \sigma^2}
\]

where \( V \) is the amplitude of the ERG b-wave elicited by a stimulus of intensity \( I \), \( V_{\text{max}} \) is the maximum response amplitude, \( n \) is a constant that determines the slope of the curve, and \( \sigma \) is the light stimulus energy needed to elicit a response of half-maximum amplitude (\( \frac{1}{2} V_{\text{max}} \)) and is therefore also termed the semisaturation constant. Since \( n \) is usually close to 1 for the dark-adapted ERG b-wave, we used \( n = 1 \) for all curve fitting.

Functional damage of the rod system in the experimental eye was assessed from the dark-adapted b-wave \( V_{\text{max}} \) ratio (experimental eye/control eye). In the light-adapted state, we could not derive the maximum amplitude of the ERG b-wave from the response-log stimulus energy relationship, because our ERG recording system did not have a sufficient range of light energies. Therefore, the functional integrity of the cone system was assessed from the light-adapted b-wave amplitude ratios (experimental eye/control eye), which were obtained from ERG responses, elicited by light stimuli at the highest available energy.

**Histologic Examination**

The enucleated eyes were soaked for 10 minutes in a solution of 4% paraformaldehyde in 0.1 M of phosphate buffer (pH 7.4). Then, the eyeball was opened posteriorly to the limbus and fixed in the same solution for 1 hour. The lens and vitreous were removed, and the posterior eye cup was cut in half. One half was rinsed in PBS, dehydrated in alcohol, soaked in a solution of resin and catalyst without the hardener overnight, and embedded in resin (JB-4; Bio-Rad, Watford City, UK). The tissue was cut into 2-μm sections (Reichert-Jung, Nussloch, Germany) and mounted on slides. The sections were stained with Richardson’s solution for examination with the light microscope.

The second half of the eye cup was washed in 0.1 M PBS solution, cryoprotected, embedded in OCT, and cut into 16-μm-thick sections along the vertical meridian on a cryostat. The cryostat sections were immunostained for glial fibrillary acidic protein (GFAP), a marker of Müller cell activation. The sections were soaked in PBS (0.1 M, pH 7.4) and then incubated in normal nonimmune serum (5% serum + 0.1% TritonX-100+PBS 0.1 M). Then, the sections were soaked separately overnight at 4°C in a moist chamber with primary antibody to GFAP (Chemicon, Temecula, CA) at 1:400 dilution in PBS 0.1 M+3% serum +0.1% TritonX-100 at 1:100.

For immunofluorescence visualization, the slides were rinsed three times in PBS and then incubated for 1 hour in donkey anti-mouse AlexaFluor 59-labeled antibody (Molecular Probes, Eugene, OR) at 1:100 in the above solution. The slides were also stained with DAPI (1:1000) to allow visualization of the cells' nuclei. The stained retinal section was observed with a fluorescence microscope (Carl Zeiss Meditec, Oberkochen, Germany).

**Statistical Analysis**

The ERG parameters \( V_{\text{max}} \) of the dark-adapted ERG b-wave and the light-adapted b-wave amplitude were tested for statistical significance by using the mixed model for analysis of variance (ANOVA). This test is more suitable to use compared with the classic ANOVA when statistical analysis of cases with repeated measures is needed and some of the data are missing. When comparing histologic data obtained at the termination of the follow-up period, data from the experimental eye and control eye were compared by Student's paired t-test.

**Results**

**Clinical Observation**

No signs of inflammation were observed after intravitreal injection and throughout the follow-up period. The cornea, the lens, and the vitreous appeared clear, and the fundus was normal in all eyes.

**Electroretinogram**

Representative ERG recordings and response-stimulus energy curves are shown for one rabbit of the low-dose group and one rabbit of the high-dose group in Figure 1. For each rabbit, the ERG responses elicited by the brightest white light stimuli (log \( I = 0.76 \) cd·s/m²) are shown in the top panel and the corresponding response-stimulus energy relationship in the bottom panel. In each pair of ERG traces, the response of the experimental eye is compared with that of the control eye. The rabbit from the low-dose group (Fig. 1a) exhibited a transient, mild reduction in the ERG of the experimental eye at the 3-hour recording session (Fig. 1a, left column) that was followed by complete recovery with time, as evident from the responses to
bright light stimuli and from the b-wave response–stimulus energy curves.

In contrast, the rabbit belonging to the high-dose group (Fig. 1b) exhibited a marked decrease in ERG responses of the experimental eye compared with the control eye that is evident in the responses to the brightest stimuli (Fig. 1b, top panel) and in the dark-adapted b-wave response–stimulus energy relationships (Fig. 1b, bottom panel). The strongest cefuroxime effect was seen shortly (3 hours) after injection, but despite partial recovery with time, a permanent ERG deficit was evident even 4 weeks after injection.

ERG recordings, similar to those shown in Figure 1, were obtained in all rabbits of both groups. In each recording session, the dark-adapted response–stimulus energy relationship of the ERG b-wave was constructed and fitted to equation 1 to derive the maximum b-wave amplitude ($V_{\text{max}}$). For each rabbit in each ERG recording session, the dark-adapted b-wave $V_{\text{max}}$ ratio (experimental eye/control eye) and the light-adapted amplitude ratio (experimental eye/control eye) of the b-wave were calculated, as described in the Methods section, and averaged.

Figure 2 summarizes the mean ± SD of the dark-adapted b-wave $V_{\text{max}}$ ratio (Fig. 2a) and the light-adapted b-wave amplitude ratio (Fig. 2b) for the low- and high-dose groups. For simplicity, only the positive parts of the standard deviation are shown. The ERG data of the low-dose group indicate a mild, but statistically significant ($P < 0.05$), early (3 hours after injection) decrease in the mean maximum dark-adapted b-wave $V_{\text{max}}$ ratio and in the mean light-adapted b-wave amplitude ratio. This was a transient effect, and a complete recovery was seen during the next 4 days of follow-up that lasted for the entire follow-up period (4 weeks). The ERG recordings of rabbits belonging to the high-dose group showed a marked early decrease in the dark-adapted b-wave $V_{\text{max}}$ ratio (approximately 73%) and in the light-adapted amplitude ratio (approximately 84%). A partial but statistically significant ($P < 0.05$)
recovery was observed with time of follow-up, but significant
(P < 0.01) permanent damage was evident even after 4 weeks
of follow-up. The permanent ERG deficit was expressed as
approximately a 27% reduction in the dark-adapted ERG b-wave
V_{max} ratio and approximately a 42% reduction in the
light-adapted b-wave amplitude ratio.

The ERG data shown in Figure 2 indicate large variability
between individual animals undergoing the same treatment
(large SD), probably reflecting variability in the degree of
retinal damage between rabbits treated with the same dose of
cefuroxime. This suggestion is supported by comparing in
Figure 3 the light-adapted b-wave amplitude ratio to the dark-
adapted b-wave V_{max} ratio for each rabbit treated with the high
and low doses of cefuroxime. These data, recorded at termina-
tion of the follow-up period (at least 2 weeks after injection)
exhibit direct correlation between the two ERG parameters.

Thus, a large degree of retinal damage was reflected in severe
deficit of retinal function for both scopic and photopic sys-
tems. When only a mild degree of retinal damage occurred,
which was mainly seen with low-dose cefuroxime, but also in
some animals treated with the high-dose cefuroxime, the sco-
topic and photopic retinal systems showed little deterioration.
The deviation of the data points in Figure 3 from the identity
line (dotted line) for large ERG deficits (low ratios) most likely
reflect uncertainties in measuring the low-amplitude, light-
adapted ERG responses compared with a better resolution
when measuring the relatively higher dark-adapted ERG re-
sponses.

The ERG responses of the experimental eye in the rabbits
injected with the high dose of cefuroxime were characterized
by an electronegative pattern. The b-wave was reduced to a
larger extent compared with the a-wave. The ERG a-wave and
b-wave reflect the algebraic summation of the photoreceptors
component (the negative P-III) and the postreceptor compo-
nent (the positive P-II). Therefore, analysis of the b-wave
to a-wave relationship can be used to localize site/s of drug-
induced damage. For instance, a selective toxic effect to post-
photoreceptor neurons and/or blockage of synaptic transmis-
sion from photoreceptors to postphotoreceptor neurons will
lead to a selective reduction in the ERG b-wave and a concom-
itant increase in the amplitude of the ERG a-wave.

To test the effects of cefuroxime on the relationships be-
tween the ERG b-wave and the ERG a-wave, we plotted the
b-wave amplitude as a function of the a-wave amplitude for all
the ERG responses that were elicited by bright light stimuli and
were characterized by measurable a-waves. This analysis,
shown in Figure 4, was applied to all ERG data at each record-
ing time interval for both doses.

The b-wave to a-wave relationship of the experimental eyes
in the rabbits in the low-dose group (Fig. 4a) was slightly
subnormal compared with that of the control eyes during the
first 4 days after injection. However, with continued follow-up,
the b-wave to a-wave relationship of the experimental eye
improved and was found to be very similar to that of the
control eye. In the high-dose group (Fig. 4b), shortly (3 hours)
after injection, the b-wave amplitudes of the experimental eyes
were significantly lower than expected from the a-wave ampli-
tudes based on the b-wave to a-wave relationship of the control
eyes (Fig 4b, top left panel). During the follow-up period,
significant improvement was observed in the b-wave to a-wave
relationship of the experimental eyes, and at 4 weeks of follow-
up, the b-wave to a-wave relationship of the experimental eyes
was very similar to that of the control eyes. The wide spread of

![Figure 2](http://tvst.arvojournals.org/...)

**Figure 2.** Time-dependent changes in the average \( \pm \) SD of the dark-adapted ERG b-wave \( V_{max} \) ratio (experimental eye/control eye) (a) and the average \( \pm \) SD of the light-adapted ERG b-wave amplitude ratio (experimental eye/control eye) (b) are shown for the low-dose and high-dose groups of rabbits during the entire 4 weeks of follow-up.

![Figure 3](http://tvst.arvojournals.org/...)

**Figure 3.** The relationship between the cefuroxime-induced reduction in scopic retinal function, as assessed from the dark-adapted b-wave \( V_{max} \) ratio, and the reduction in photopic retinal function, as assessed from the light-adapted b-wave amplitude ratio. Data points represent retinal function at termination of the follow-up period of rabbits injected with low-dose and high-dose cefuroxime. Dotted line: identity.
The data points did not allow accurate assessment of whether the two relationships were similar or different.

**Histology**

Representative histologic micrographs of one rabbit from each dose group are shown in Figure 5. No histologic damage was evident in the retina of the rabbit injected with the low dose of cefuroxime (Fig. 5a). The retina from the experimental eye retained its normal layered structure in regions close to the injection site and in remote loci. Similar observations were seen in a total of six rabbits belonging to the low-dose group that were tested for structural retinal damage.

The experimental retina from the rabbit treated with the high dose of cefuroxime (Fig. 5b) showed marked damage when compared with the retina of the control eye. Retinal damage was most apparent in the inferior retina probably reflecting our attempt to inject the drug in the region of the optic disc and the spread of the drug down toward the inferior retina (Fig. 5b, top left panel). Evidence of the damage was loss of the retinal layered structure, disorganization, and retinal thinning. Remote retinal loci in the inferior retina and most of the superior retinal regions of the experimental eye treated with the high-dose cefuroxime appeared to be normal (Fig. 5b, bottom row).

Histologic findings, similar to those presented in Figure 5b, were observed in eight rabbits, belonging to the high-dose group, but the magnitude of retinal structural damage differed between rabbits. Variability in the degree of cefuroxime-induced damage was expected because the intravitreal injection of the drug could not be identical between different animals treated at different times.

To assess the degree of cefuroxime-induced retinal structural damage, we measured the total length of damaged retina in one section of the experimental eye in each of the eight rabbits and compared it in Figure 6a to its dark-adapted b-wave \( V_{\text{max}} \) ratio. Despite technical difficulties in length measurements and the small sample size (\( n = 8 \)), the trend is clear: the larger the region of retinal damage the larger the degree of ERG deficit, as represented by a lower dark-adapted b-wave \( V_{\text{max}} \) ratio. The relationship between retinal damage defined functionally from the ERG and the length of damaged retina fits quite well to a linear equation (\( R = 0.94 \)), supporting the notion that the ERG responses reflect algebraic summation of contributions by small retinal regions.

We also measured retinal thickness, from the vitreoretinal junction to the tips of the photoreceptor outer segments in the control eyes and experimental eyes of both groups of rabbits. Thickness measurements were obtained in the inferior retina at a distance of 2 mm from the optic disc, a region that was consistently damaged in the eyes exhibiting cefuroxime-induced damage. In the low-dose group (\( n = 6 \)), we measured an average (±SD) retinal thickness of 168.28 ± 4.15 μm and 170.56 ± 8.83 μm in the experimental and control eyes, respectively. These values did not differ significantly (\( P = 0.360 \)), as determined by Student’s paired \( t \)-test. In the high-dose group (\( n = 7 \)), a trend was seen toward lower retinal thickness of the experimental eyes compared with the control eyes, but the average (±SD) retinal thickness of the experimental eyes, 138.93 ± 50.42 μm was not significantly different (\( P = 0.059 \)) from that of the control eyes, 164.06 ± 24.19 μm. We attribute this observation to the large variability in the degree of retinal damage, as expressed by the large standard deviation of the measurements. To test this possibility, we compared the relationship between the degree of functional damage, as assessed from the dark-adapted b-wave \( V_{\text{max}} \) ratio, and the retinal thickness ratio (experimental eye/control eye) in seven rabbits from the high-dose group (Fig. 6b). The data show some variability, but clearly indicate that the larger the degree of structural damage (lower retinal thickness ratio), the larger the degree of ERG damage (lower dark-adapted b-wave \( V_{\text{max}} \) ratio). It should be noted that since we compared the ERG deficit to total retinal thickness, a nonrecordable ERG was expected for a retinal thickness of more than 0.

Glial fibrillary acidic protein (GFAP) is an intermediate filament that is normally expressed in astrocytes but not in Müller cells in the retina.\(^{20}\) However, in a variety of retinal injuries including retinal detachment,\(^{21}\) ischemia,\(^{22}\) and increased intraocular pressure,\(^{23}\) GFAP expression in retinal Müller cells became apparent. Therefore, GFAP expression in Müller cells is widely used as a molecular indicator for retinal stress.

Representative micrographs of experimental and control retinas of one rabbit from each dose group are shown in Figures 7.
GFAP expression in Müller cells is evident in the experimental retinas from the rabbits in both dose groups (Fig. 7, top panels), but not in the control retinas (Fig. 7 bottom panels). The expression of GFAP in Müller cells was more extensive in the retina exposed to the high dose of cefuroxime (Fig. 7 top right panel) compared to that of the retina exposed to the low dose of the drug (Fig. 7, top left panel). In the retinas of the experimental and control eyes of both groups, GFAP immuno-

**Figure 5.** Representative micrographs of retinal sections from a rabbit treated with the low-dose cefuroxime (a) and from another rabbit treated with high-dose cefuroxime (b). For each rabbit, sections from an area in the inferior retina, close to the site of injection, and from a remote site in the inferior retina of the experimental eye and of the control eye are shown. ONL, outer nuclear layer; INL, inner nuclear layer; GCL, ganglion cell layer.

**Figure 6.** Comparing cefuroxime-induced functional damage (ERG responses) to structural damage (histologic measurements). (a) Dependence of the degree of cefuroxime-induced ERG deficit, assessed from the dark-adapted b-wave $V_{\text{max}}$ ratio, on the degree of cefuroxime-induced structural damage, assessed from a length of damaged retina. Comparison was made for eight rabbits that were treated with high-dose cefuroxime. The data were fitted to a linear function (dashed line). (b) Relationship between the dark-adapted b-wave $V_{\text{max}}$ ratio and the retinal thickness ratio, measured 2 mm inferior to the optic nerve, for seven rabbits treated with high-dose cefuroxime.
Our results indicate that cefuroxime exerts dose-dependent transient and permanent effects on the rabbit retina after intravitreal administration of the drug: the higher the dose of the drug, the larger the transient and permanent effects, as indicated by the direct relationship between them (Fig. 3). However, within a few days the ERG parameters completely recovered, and no apparent ERG deficit was observed after 4 weeks of follow-up (Figs. 2a, 4a). The high dose of cefuroxime induced a severe ERG deficit in the dark-adapted and light-adapted responses that was most apparent shortly (3 hours) after the injection. Despite gradual and statistically significant recovery with time of follow-up, a permanent, statistically significant ERG deficit was evident at the end of the 4-week follow-up period indicating damage to the distal retina (Fig. 2b). The average degree of permanent cefuroxime-induced ERG deficit was approximately 27% in the dark-adapted ERG and approximately 42% in the light-adapted ERG. Despite the large variability in the ERG responses of the high-dose group, the dark- and light-adapted ERGs seemed to be equally affected, as indicated by the direct relationship between them (Fig. 3).

The ERG findings were supported by the histologic observations (Fig. 5). The retinas from the eyes treated with low-dose cefuroxime retained a normal layered structure and appeared very similar to the retinas from the control eyes of the same rabbits throughout the entire retinal length. We did not observe any localized retinal areas exhibiting any visible structural damage, and the total retinal thickness at a site 2 mm inferior to the optic disc, a site that was always damaged in the rabbits treated with high-dose cefuroxime, did not differ between the experimental eye and control eye of the rabbits belonging to the low-dose group. In contrast, retinas from the eyes that were treated with the high dose of cefuroxime showed mild to severe structural damage that was expressed as a loss of photoreceptor outer segments, disorganization of the layered retinal structure and retinal thinning. The damaged area was always observed in the inferior retina, roughly the region of drug injection. Also, the drug might have tended to diffuse more toward inferior retinal regions due to the rabbits’ head posture.

The magnitude of cefuroxime-induced histologic damage, as assessed from the length of damaged retina and from retinal thickness at a site close to the site of injection, could account for the variability in the cefuroxime-induced ERG deficit (Figs. 6a, 6b, respectively). The dark-adapted b-wave $V_{\text{max}}$ ratio is inversely related to the length of damaged retina (Fig. 6a), and directly related to retina thickness ratio (Fig. 6b), indicating that when cefuroxime induces larger degree of structural damage, the ERG of the experimental eye is smaller. The variability in the cefuroxime-induced damage probably reflects technical variability in the injection procedure between animals studied in different experimental days: the closer the injecting needle to the retina, the larger the degree of damage. These observations clearly demonstrate the dependency of toxic effects on technical variability of intravitreal injection and support electrophysiological and structural analysis of individual animals in addition to group assessment.

In contrast to the clear ERG and histologic differences between rabbits administered low-dose cefuroxime and those treated with the high dose, rabbits from both groups (two from the low-dose group and four from the high-dose group) were very similar when their retinas were tested for GFAP immunostaining (Fig. 7). In both retinas, GFAP immunoreactivity was evident in retinal Müller cells, but the extent of GFAP immunostaining was more prominent in Müller cells of retinas exposed to high-dose cefuroxime compared with retinas exposed to the low dose. It should be noted that the retinal sections shown in Figure 7 were obtained from the inferior retina, but were sufficiently remote from the site of injection that they sustained minimal damage, as was evident from the layered organization and the thickness of the outer and inner nuclear layers and the ganglion cell layer.

**DISCUSSION**

The finding of GFAP expression in Müller cells of the retinas exposed to high-dose cefuroxime was expected, considering the functional (ERG responses) and histologic damage that was found. However, GFAP expression in the Müller cells of the retinas exposed to the low dose of cefuroxime was not expected, considering the normal ERG responses and histologic findings at the end of follow-up.

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The low dose of cefuroxime showed a very mild, early effect on retinal function, as indicated by a slight reduction (~25%) in the ERG responses and an electronegative pattern in which the b-wave was more affected than the a-wave (Fig. 4a). However, within a few days the ERG parameters completely recovered, and no apparent ERG deficit was observed after 4 weeks of follow-up (Figs. 2a, 4a). The high dose of cefuroxime induced a severe ERG deficit in the dark-adapted and light-adapted responses that was most apparent shortly (3 hours) after the injection. Despite gradual and statistically significant recovery with time of follow-up, a permanent, statistically significant ERG deficit was evident at the end of the 4-week follow-up period indicating damage to the distal retina (Fig. 2b). The average degree of permanent cefuroxime-induced ERG deficit was approximately 27% in the dark-adapted ERG and approximately 42% in the light-adapted ERG. Despite the large variability in the ERG responses of the high-dose group, the dark- and light-adapted ERGs seemed to be equally affected, as indicated by the direct relationship between them (Fig. 3).

The ERG findings were supported by the histologic observations (Fig. 5). The retinas from the eyes treated with low-dose cefuroxime retained a normal layered structure and appeared very similar to the retinas from the control eyes of the same rabbits throughout the entire retinal length. We did not observe any localized retinal areas exhibiting any visible structural damage, and the total retinal thickness at a site 2 mm inferior to the optic disc, a site that was always damaged in the rabbits treated with high-dose cefuroxime, did not differ between the experimental eye and control eye of the rabbits belonging to the low-dose group. In contrast, retinas from the eyes that were treated with the high dose of cefuroxime showed mild to severe structural damage that was expressed as a loss of photoreceptor outer segments, disorganization of the layered retinal structure and retinal thinning. The damaged area was always observed in the inferior retina, roughly the region of drug injection. Also, the drug might have tended to diffuse more toward inferior retinal regions due to the rabbits’ head posture.

The magnitude of cefuroxime-induced histologic damage, as assessed from the length of damaged retina and from retinal thickness at a site close to the site of injection, could account for the variability in the cefuroxime-induced ERG deficit (Figs. 6a, 6b, respectively). The dark-adapted b-wave $V_{\text{max}}$ ratio is inversely related to the length of damaged retina (Fig. 6a), and directly related to retina thickness ratio (Fig. 6b), indicating that when cefuroxime induces larger degree of structural damage, the ERG of the experimental eye is smaller. The variability in the cefuroxime-induced damage probably reflects technical variability in the injection procedure between animals studied in different experimental days: the closer the injecting needle to the retina, the larger the degree of damage. These observations clearly demonstrate the dependency of toxic effects on technical variability of intravitreal injection and support electrophysiological and structural analysis of individual animals in addition to group assessment.

In contrast to the clear ERG and histologic differences between rabbits administered low-dose cefuroxime and those treated with the high dose, rabbits from both groups (two from the low-dose group and four from the high-dose group) were very similar when their retinas were tested for GFAP immunostaining (Fig. 7). In both retinas, GFAP immunoreactivity was evident in retinal Müller cells, but the extent of GFAP immunostaining was more prominent in Müller cells of retinas exposed to high-dose cefuroxime compared with retinas exposed to the low dose. It should be noted that the retinal sections shown in Figure 7 were obtained from the inferior retina, but were sufficiently remote from the site of injection that they sustained minimal damage, as was evident from the layered organization and the thickness of the outer and inner nuclear layers and the ganglion cell layer.
GFAP immunostaining serves as a molecular marker of Müller cell reactivity triggered by retinal stress of various etiologies, but it has also been reported that GFAP may be overexpressed in Müller cells after retinal stress, even if normal histology is found and no sign of cellular degeneration is seen. We therefore suggest that GFAP immunostaining can be a more sensitive tool for detecting retinal stress than either the ERG recordings or histologic observations at the light microscopy level. Thus, the low dose of cefuroxime probably induced sufficient retinal stress to cause activation of retinal Müller cells, but insufficient to be expressed either in the ERG responses or in histologic observations at the light microscopy level.

The most striking effect of cefuroxime on the ERG responses was the transient selective reduction of the ERG b-wave that was sometimes accompanied by augmentation of the ERG a-wave, leading to development of a typical electrogeneative pattern of the ERG responses. This was most apparent in the eyes injected with the high dose of cefuroxime when examined shortly after injection (Figs. 2b, 4b).

An electrogeneative pattern of ERG responses usually reflects abnormal signal transmission from photoreceptors to ON-center bipolar cells that can result from either a block in synaptic transmission and/or abnormal functioning of the ON-center bipolar cells. Antibiotics from the β-lactam family, cefuroxime included, were found to promote overexpression of i-glutamate transporter 1 (GLT1) in the central nervous system. The dark-adapted concentration of i-glutamate in the outer plexiform layer of the vertebrate retina reflects the balance between i-glutamate release by the photoreceptors and i-glutamate removal by i-glutamate transporters; GLAST1 in Müller cells and GLT1 in photoreceptors and second-order neurons. We suggest that cefuroxime-induced GLT1 overexpression in the distal retinal neurons lowers i-glutamate concentration in the synapses of the outer plexiform layer, leading to depolarization of ON-center bipolar cells. Therefore, the light-induced electrical activity of these neurons is expected to be reduced in amplitude. Since the ERG b-wave reflects mainly light-induced activity of ON-center bipolar cells, the net result is a selective reduction of the ERG b-wave leading to an ERG of electrogeneative pattern. As cefuroxime is cleared from the vitreous, GLT1 levels return to normal, and the ERG responses recover in amplitude and attain a normal pattern of b-wave to a-wave relationship. Thus, we suggest that cefuroxime can induce a transient physiological effect on synaptic transmission from photoreceptors to second-order neurons lasting as long as cefuroxime is present in the retina. This physiologic effect can result in a temporary disturbance in vision. In addition, cefuroxime can cause toxic effects, mediated by yet unidentified mechanisms. These effects are permanent and may cause a longlasting disturbance in vision.

Koul et al. found that intravitreal injection of cefuroxime at a dose of 1 mg in rabbit eyes caused no damage to the rabbit retina, as determined by ERG and histology. These histologic findings correspond to our long-term findings with the same antibiotic, and no sign of cellular degeneration is seen. However, we also observed a mild, transient ERG deficit (Fig. 1a) and glial activation (GFAP expression in Müller cells), indicating retinal stress (Fig. 7). These differences probably reflect difference in the group size that was used in both studies (n = 2 in Koul et al. versus n = 9 in the present study) and different indicators for retinal stress that were used.

Our findings are consistent with previous reports of retinal damage after exposure to different cephalosporins. The dark-adapted ERG b-wave was shown to be affected after an intravitreal injection of cephaloridine. Certain cephalosporins (cephaloridine, cefotetan, and ceftazidime) were found to induce dose-dependent histologic damage that was primarily expressed in the photoreceptors. Recently, clinical reports of cefuroxime ocular toxicity due to inadvertent intracameral injection of high doses of cefuroxime have been published. One of these reports describes a series of six cases in which 40 to 50 mg cefuroxime was injected intracameraly after successful phacoemulsification cataract surgery. Optical coherence tomography revealed extensive macular edema with a large serous retinal detachment followed by improvement during a 6-week follow-up. Fluorescein angiograms showed diffuse leakage. The macular thickness and profile returned to normal in all patients, although ERG recordings showed reduced rod photoreceptor function. The final visual outcome was satisfactory in all six cases.

We report that a cefuroxime dose (10 mg/0.1 mL) that is 10 times larger than the dose used in clinical practice (1 mg/0.1 mL) caused retinal damage after administration into the rabbit vitreous. In contrast, the cefuroxime dose that is commonly used in clinical practice for intracameral injection (1 mg/0.1 mL) did not cause any detectable damage to the rabbit retina when injected into the vitreous. Since the rabbit eye is smaller than the human eye and has a vitreous volume approximately two thirds that of the human, the dose of 1 mg/0.1 mL was produced larger than inadvertent injection of the same dose into the human eye. Furthermore, because of the retinal vascular system of the human eye, the rate of drug clearance from the vitreous is probably faster than in the rabbit eye, with minimal retinal vasculature. Therefore, it is quite safe to say that the dose of 1 mg/0.1 mL, commonly used in clinical practice for intracameral injection, is safe even if it is inadvertently injected into the vitreous. In contrast, a 10-fold larger dose of the drug (10 mg/0.1 mL) caused significant damage to the rabbit retina.

It is difficult to extrapolate from albino rabbit studies to human eyes. However, considering the results of this study and the reports on cefuroxime toxicity in patients, it is advisable to be careful with the intraocular cefuroxime dose and the locus of injection.

References

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