Safety of Intravitreally Injected Ciprofloxacin in Phakic Rabbit Eyes

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This study was designed to determine the maximal safe drug concentration of intravitreal ciprofloxacin in phakic rabbit eyes. Twenty-two eyes of New Zealand pigmented rabbits received midvitreal ciprofloxacin of 100, 200, 400, 600 or 800 μg in BSS Plus, or BSS Plus only. Retinal toxicity was dose-dependent as determined with electroretinography, light microscopy, and transmission electron microscopy. At a dose of greater than 400 μg, disorganization of the outer segments was a main pathological finding in transmission electron microscopy. We evaluated retinal function by measuring the electroretinograms for a graded series of flash intensities and by fitting electroretinogram b-wave amplitudes to the Naka-Rushton equation. At a dose of greater than 600 μg, Rmax was significantly decreased and log K was significantly increased. N-value tended to decrease. A decrease of b-wave amplitudes caused by retinal toxicity could be detected very sensitively with lower luminance stimuli. Determination of retinal toxicity with lower luminance electroretinography revealed a significant decrease of b-wave amplitudes at a dose of greater than 400 μg. We concluded that a safe dose of intravitreal ciprofloxacin in phakic rabbit eyes was 200 μg in phakic eyes.

Key words: intravitreal ciprofloxacin, electroretinogram, Naka-Rushton equation parameters, electron microscopy, retinal toxicity

INTRODUCTION

Bacterial endophthalmitis is a devastating complication of intraocular surgery and eye trauma. Because of poor intraocular penetration of topicaly, subconjunctivally, sub-Tenon and systematically administered antibiotics, bacterial endophthalmitis continues to pose a major challenge to the ophthalmologist. Intravitreal antibiotics are critical for the successful treatment of bacterial endophthalmitis.1–6 In endophthalmitis associated with virulent organisms, attempts have included intravitreal antibiotics after total vitrectomy. Combining their use with vitrectomy remains controversial because of the lack of controlled, randomized clinical trials describing the effects of such a combination.5

Increased antibiotic resistance among organisms associated with bacterial endophthalmitis necessitates the evaluation of newer, more potent antibiotics. Ciprofloxacin, a broad-spectrum antibiotic, acts as a bactericidal agent by inhibiting DNA gyrase,7,8 and has been effective against multiple resistant organisms, including Pseudomonas aeruginosa and Enterobacteriaceae.7–11 Intensive studies were undertaken by several authors to determine a nontoxic dose of ciprofloxacin for intravitreal injection and its clinical application.12–15 In some studies, results of electroretinography and light microscopy alone
were insufficient for evaluating the retinal toxicity of ciprofloxacin.\textsuperscript{13}

In this study, ciprofloxacin monotherapy was evaluated intraocularly in phakic rabbit eyes to determine a drug concentration that could be used without detectable ocular toxicity electroretinographically and histopathologically. This study differed from the majority of research works in that we used a wide range of flash intensities rather than the single intensity electroretinogram (ERG) to specify an alteration in retinal function much more clearly. The multiple intensity ERG data for determining the three parameters of Naka-Rushton equation ($R_{\text{max}}$, log K, and n) were analyzed.

\section*{MATERIALS AND METHODS}

\section*{Animals}

Twenty-four eyes of 16 New Zealand pigmented rabbits weighing about 2.5 kg were used in this study. The animals underwent a complete clinical examination that included slit-lamp biomicroscopy, indirect ophthalmoscopy and tonometry.

\section*{Intravitreal Ciprofloxacin}

The pupil was dilated with topically-applied 1\% tropicamide and the rabbit was anesthetized with urethane intravenous bolus (400 mg/kg) followed by an intravenous drip infusion (200 mg/kg/hr). A 26-gauge needle on a tuberculin syringe was introduced through the sclera adjacent to the fixation forceps 3 mm posterior to the limbus. The eyes were injected with ciprofloxacin (Cycin 100mg/50 ml, Ildong Pharm. Co., Korea) after dilution was made with BSS-Plus in a dose of 100 µg, 200 µg, 400 µg, 600 µg, or 800 µg per 0.1 mL and BSS-Plus only. Each group consisted of 4 eyes. The needle was guided to the approximate center of the vitreous body, and 0.1 mL of solution was slowly injected. Ethical considerations preclude a simultaneous potentially blinding dose of antibiotic injection on both eyes. One eye of the animal thus received a dose greater than 200 µg ciprofloxacin and the other eye received BSS Plus only or a dose less than 100 µg.

\section*{Electrophysiological Examinations}

The rabbit was dark-adapted for 60 minutes. An ERG jet contact lens electrode (Universo Sa, Switzerland) was connected to a cathode ray oscilloscope (VC-10, Nihon Koden, Tokyo) through a biophysical amplifier (AVB-11, Nihon Kohden). A ground and reference electrode was placed on each earlobe. The above ERG preparation was accomplished in dim red light to minimize any light adaptation. Neuroelectrical signals were amplified with a bandpass of 0.5 Hz to 1 kHz. Stimuli were white full-field flashes delivered by xenon strobe, presented in a commercial dome (Model 7310, Life-Tech Instrument Inc, Houston, Texas). Single-flash ERGs were recorded at stimulus luminances ranging from -4.5 log cd s/m² to 0 log cd s/m² in 0.5 log unit step with Wratten neutral density filter, and presented in ascending order from dimmest to brightest. The repetition interval of the stimulus was 15 sec. The stimulus strength and duration were measured by a Tektronix J-16 digital photometer and a Tektronix 314 storage oscilloscope. The stimulus luminance of 0 log cd · s/m² was 2.2 cd · s/m² and its duration 23 msec. At low luminances the ERG was averaged from 4 to 12 times, depending on noise level. The amplitude of the b-wave was measured according to convention from the trough of the a-wave to the peak of the b-wave.

\section*{Electrophysiological Data Analysis}

The ERG waveforms were examined primarily for amplitude informations. The flash intensity and ERG b-wave amplitude values for the points were used to derive the parameter of the Naka-Rushton equation\textsuperscript{16}: $R = R_{\text{max}} I^N/(I^N + K^N)$

In this equation, $I$ is the stimulus luminance of the flash and $R$ is the ERG b-wave amplitude. $R_{\text{max}}$ is the asymptotic ERG amplitude. It represents the total area of the functioning retina. $K$ is the half-saturation constant. It corresponds to retinal sensitivity. $N$ is a dimensionless constant controlling the slope of the function. It represents the degree of heterogeneity of retinal sensitivity.

\section*{Tissue Processing}

On day 14 after the intravitreal injection, experimental animals were sacrificed by intravenous
overdose of sodium pentobarbital through the marginal ear vein. Within seconds of enucleation, the eyes were immersed in 2.5% glutaraldehyde while removing the anterior segment and vitreous. The tissues were immersed in 2.5% glutaraldehyde. Blocks of the tissue were prefixed in 2.5% glutaraldehyde for 2 hours at 4°C and postfixed in 1% osmium tetroxide in 0.1 M phosphate buffer for 2 hours at room temperature. The specimens were dehydrated in graded ethanol and embedded in an epon mixture. One micrometer sections were stained with hematoxylin-eosin for a critical determination of the anatomical orientation. After ultrathin sectioning with Porter-Blum MT-2B ultramicrotome and staining with uranyl acetate and lead citrate, the tissues were examined with a Jeol JEM 100-CX electron microscope.

RESULTS

Electrophysiological Findings

We were mainly interested in the b-wave amplitude, the decrease of which was dose-dependent. Electroretinographic results revealed no loss of the b-wave amplitudes with tested intravitreal BSS Plus compared with preinjection baseline results. The post-drug ERGs performed on day 7 after the intravitreal ciprofloxacin injection showed statistically no significant decrease of the b-wave amplitudes in all 5 groups of experimental eyes (both the t-test and NPARIWAY procedure). However, a decrease of the average b-wave amplitudes was 16.2-30.6% at a dose of ciprofloxacin 400 μg, 25-42.9% at 600 μg, and 40-43.6% at 800 μg between lumenance intensities of \(-\log 2.0\) to \(-\log 4.5\) cd · s/m². Some authors indicate that a difference in amplitude of the same eye on different occasions is probably pathologic if it is between 20% and 24% and highly likely to be pathologic if it exceeds 24%.22 A reduction in ERG amplitude during serial follow-up examinations is significant, at the 99% confidence limit, if a decline of greater than 31% occurs from a single-flash white stimulus.23 The post-drug ERGs performed on day 14 after intravitreal ciprofloxacin showed consistently decreased b-wave amplitudes at a dose greater than 400 μg and these values were statistically significant. A decrease of b-wave responses was very sensitive to lower luminance.

**Table 1.** Rmax, n and logK derived from the Naka-Rushton equation curve fit, as an average value (mean ± 1SD) in control and ciprofloxacin treated eyes

<table>
<thead>
<tr>
<th></th>
<th>Rmax</th>
<th>n</th>
<th>logK</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSS+</td>
<td>BV</td>
<td>350.0 ± 42.4</td>
<td>0.86 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>1W</td>
<td>347.0 ± 41.9</td>
<td>0.83 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>2W</td>
<td>350.0 ± 42.4</td>
<td>0.85 ± 0.08</td>
</tr>
<tr>
<td>100 mg</td>
<td>BV</td>
<td>325.0 ± 17.3</td>
<td>0.86 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>1W</td>
<td>325.0 ± 10.0</td>
<td>0.82 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>2W</td>
<td>325.0 ± 10.0</td>
<td>0.84 ± 0.06</td>
</tr>
<tr>
<td>200 mg</td>
<td>BV</td>
<td>410.0 ± 127</td>
<td>0.89 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>1W</td>
<td>367.5 ± 49.9</td>
<td>0.90 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>2W</td>
<td>370.0 ± 47.6</td>
<td>0.89 ± 0.04</td>
</tr>
<tr>
<td>400 mg</td>
<td>BV</td>
<td>482.5 ± 38.6</td>
<td>0.86 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>1W</td>
<td>450.0 ± 73.9</td>
<td>0.83 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>2W</td>
<td>400.0 ± 84.8</td>
<td>0.79 ± 0.16</td>
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<tr>
<td>600 mg</td>
<td>BV</td>
<td>512.5 ± 55.0</td>
<td>0.85 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>1W</td>
<td>427.5 ± 86.5</td>
<td>0.79 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>2W</td>
<td>402.5 ± 65.5*</td>
<td>0.78 ± 0.10</td>
</tr>
<tr>
<td>800 mg</td>
<td>BV</td>
<td>512.5 ± 58.5</td>
<td>0.86 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>1W</td>
<td>427.5 ± 86.5</td>
<td>0.78 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>2W</td>
<td>400.0 ± 65.5*</td>
<td>0.77 ± 0.10</td>
</tr>
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</table>

BV: base line value, W: week or weeks, *: statistically significant p < 0.05 (both the t-test an NPARIWAY procedure)
stimuli below -2.0 cd·s/m². Typical scotopic rabbit ERG records of the normal and experimental eyes for studying the luminance-response function were plotted in figure 1. The second amplitude increase or limb appeared at luminance intensity of log 0 cd·s/m². We thus took -log 0.5 cd·s/m² for Rmax. The solid line represented a fitting curve performed by Naka-Rushton equation to flash luminance below -0.5 log cd·s/m². An alteration was seen for the 600 µg and 800 µg groups with the Naka-Rushton parameters Rmax and log K (Table 1). Rmax, the asymptotic value of the ERG amplitude as a function of intensity, was decreased and may be attributed to a loss of the area of the functioning retina. Log K, the flash intensity that produces an ERG whose amplitude is one-half that of Rmax, was increased. This means a decrease of the retinal sensitivity. N-values tended to decrease, however, these values were statistically not significant (Table 1). This phenomenon may reflect relatively homogenous damage of the retina caused by intravitreal ciprofloxacin.

**Histopathological Findings**

The specimens were obtained on day 14 after intravitreal injections. Results of light microscopy showed slight toxicity in eyes injected with 800 µg of ciprofloxacin. In a survey of the retina, the hematoxylin-eosin stained epon section showed a loss of the outer segments followed by an atrophic change of the inner segments as well as the outer and inner nuclear cell layers. In some preparations, minimal perceivable degenerative changes were observed in the inner plexiform or ganglion cell layers. No evidence of retinal degeneration was found with the light microscope in the other dosegroups. Results of transmission electron microscopy (TEM) of experimental eyes injected with ciprofloxacin demonstrated evidence of significant retinal toxicity at a dose greater than 400 µg. Characteristic changes noted were disorganization of the outer segments. There were mitochondrial swelling and vacuolization in the pigment epitheliums, inner segments, bipolar cells and ganglion cells. An intravitreal dose of greater than 400 µg resulted in dose-dependent retinal toxicity in experimental eyes. There was no significant evidence of retinal toxicity on TEM that served as surgical controls or in eyes injected with 100 µg or 200 µg of intravitreal ciprofloxacin.

**DISCUSSION**

We evaluated the retinal function by measuring the ERG for a graded series of flash intensities and fitting ERG b-wave amplitudes to the Naka-Rushton equation (Figure 1). At very low or moderate luminance, a large difference of luminances causes a very small change in the response. In a mid-range of luminances the relationship is linear in a logarithmic plot. However, the b-wave luminance-response function at high luminances is not adequately described by Naka and Rushton. At high luminances, a second amplitude increase or limb

![Fig.1. ERG b-wave amplitudes plotted as a function of stimulus luminance in control eyes and eyes treated with Ciprofloxacin 800 mg. The solid curve represents the least squares fit of Naka-Rushton equation to mean values of parameters. -0.5log cd.s/m was taken for Rmax (see results and discussions) in control eyes : Rmax = 512.5 log K = -2.39 n = 0.86, in ciprofloxacin 800 mg treated eyes on day 7 : Rmax = 427.5 log K = -2.15 n = 0.78, on day 14 : Rmax = 400.0 log K = -1.76 n = 0.77](image-url)
appears in the function. We also found a second amplitude increase in the luminance-response function in rabbit. A statistical analysis of these b-wave responses are beyond the present study. We evaluated the 3 parameters described by the equation: \( R_{\text{max}}, \log K \) and \( n \). The post-drug ERGs performed on day 14 after intravitreal ciprofloxacin showed consistently decreased b-wave amplitudes with lower luminance stimuli for a dose greater than 400 \( \mu g \) and these values were statistically significant. An alteration was seen for the 600 \( \mu g \) and 800 \( \mu g \) groups with Naka-Rushton parameters \( R_{\text{max}} \) and \( \log K \) (Table 1). \( R_{\text{max}} \), the asymptotic value of the ERG amplitude as a function of intensity, was decreased and may be attributed to a loss of the area of functioning retina or shunting of the electrical potential. Log \( K \), the flash intensity that produces an ERG whose amplitude is one-half that of \( R_{\text{max}} \), was increased. This means a decrease of the retinal sensitivity. The value for log \( K \) could be evaluated as a result of decreased photopigment optical density, increased light filtering by the preretinal media, and less effective neurotransmitter formation. N-values were relatively consistent in all experimental eyes. This phenomenon may reflect homogenous damage of the retina caused by intravitreal ciprofloxacin. The reduction of value for \( n \) in the Naka-Rushton equation may occur secondary to heterogenous depression of the retinal sensitivity, uneven retinal illumination, or some putative neurophysiological response anomaly. However, some investigators assign the progressive lowering of \( n \) to abnormal photoreceptor neural processing and/or to abnormal postreceptor influences such as increased latencies, but not to the non-uniformities of retinal illumination. A correct evaluation of the parameter \( n \) is still debatable. However, we were able to look for and specify an alteration in retinal function much more clearly than in experiments that have used single flash intensity.

Results of the ERGs that used single intensity flash or limited comparison to absolute amplitudes are known insufficiently to evaluate the retinal toxicity of intravitreal antibiotics. In the present study, ERG b-wave produced with low intensities (below -2.0 log cd · s/m²) accurately reflected histopathological alterations of the retina. Two-hundreds g of intravitreally injected ciprofloxacin did not cause a significant functional depression, but at a dose of greater than 400 \( \mu g \) the low luminance ERGs showed a significant decrease of b-wave amplitudes. The TEM was the most sensitive method for determining retinal toxicity after an intravitreal injection of ciprofloxacin. There was no

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**Fig. 2.** Rabbit retina, on day 14 after intravitreal ciprofloxacin 400 mg. The pigment cells (PE) show marked swelling of mitochondria. Fine granular substances(*) representing edema are accumulated in intracellular spaces of the outer segment (OS). Mitochondria of the inner segments of rod and cells (C) are markedly swollen. Bar = 1 \( \mu m \)

**Fig. 3.** Rabbit retina, on day 14 after intravitreal ciprofloxacin 600 \( \mu g \). The pigment cells (PE) and inner segments (IS) of rod and cone cells (C) demonstrate marked swelling of mitochondria. Outer segments (OS) of photoreceptor cells are disorganized. Bar = 1 \( \mu m \)
significant evidence of retinal toxicity on TEM in phakic rabbit eyes with 200 µg of intravitreal ciprofloxacin. An evidence of significant disorganization of the outer segment was noted on TEM at a dose of 400 µg. A safe intravitreal dose of ciprofloxacin may be 200 µg in phakic rabbit eyes. Some investigators reported that TEM revealed a consistent evidence of significant retinal toxicity at an intravitreal dose of 250 µg, however at a dose of 100 µg was safe.13,14 But they did not perform animal experiments between dosage of 100 µg to 250 µg. This dosage may be safe only in phakic rabbit eyes. However it is reported that an intravitreal dose of greater than 100 µg results in acute corneal toxicity in aphakic vitrectomized rabbit eyes.13

Ciprofloxacin is effective against the most common causes of bacterial endophthalmitis, for example: Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus pneumoniae, Enterobacteriaceae, Pseudomonas aeruginosa and streptococci etc., with a minimum inhibitory concentration (MIC₉₀) level of below 2.0 mg/L in vitro experiments.13 Effective antibiotic treatment of bacterial endophthalmitis without detectable tissue toxicity requires a concentration 2-10 times the MIC₉₀. Based on the fact that the average volume of the rabbit vitreous body is about 1.4 mL, 200 µg/1.4 mL of ciprofloxacin must be an effective concentration for the treatment of endophthalmitis caused by most common bacterial species. In normal rabbit eyes the elimination half life is 2.2 hours and in aphakic vitrectomized eyes 1 hour.15 Because of the relatively short half life, intravitreal administration of ciprofloxacin for the treatment of endophthalmitis would provide only short-term therapy. Moreover, some investigators reported the poor bactericidal effect of intravitreally injected antibiotics in vivo experiments.12 This poor bactericidal effect of antibiotic may be related to transient phenotypic alterations in the bacteria in response to changes in the environment of infection such as hypoxia, low pH and the exhaustion of bacterial nutrients.12 Therefore, it seems reasonable to use the maximal nontoxic dose whenever intravitreal ciprofloxacin therapy is considered necessary.

REFERENCES


