The efficacy of intravitreal gatifloxacin and vancomycin in rabbits with experimental \textit{Staphylococcus epidermidis} endophthalmitis

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The efficacy of intravitreal gatifloxacin and vancomycin in rabbits with experimental *Staphylococcus epidermidis* endophthalmitis

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The Doctoral Dissertation submitted to the Department of Medicine, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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Soo Young Lee
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ABSTRACT

The efficacy of intravitreal gatifloxacin and vancomycin in rabbits with experimental Staphylococcus epidermidis endophthalmitis

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To determine whether gatifloxacin can be used safely as an intravitreal agent, retinal toxicity of intravitreal gatifloxacin was studied in a rabbit model. Additionally, the outcomes of intravitreal gatifloxacin were compared with those of intravitreal vancomycin in rabbit eyes with experimental Staphylococcus epidermidis endophthalmitis to assess the efficacy of the gatifloxacin.

The toxicity and efficacy of gatifloxacin were evaluated using 55 New Zealand white rabbits. In the toxicity study, twenty-five rabbits were divided into 5 groups (5 rabbits in each) and the right eyes were treated with five intravitreal
doses of gatifloxacin (1000, 500, 250, 100, and 50 μg/0.1 mL). The left eyes served as controls and were injected with 0.1 mL of balanced salt solution. The treatment effect of 200 μg/0.1 mL of intravitreal gatifloxacin in the experimental *S. epidermidis* endophthalmitis was compared with that of 1000 μg/0.1 mL of intravitreal vancomycin and intravitreal balanced salt solution (untreated control) using 30 rabbits. Intravitreal antibiotic therapy commenced 24 h after *S. epidermidis* intravitreal challenge (10⁵ colony forming unit/0.1 mL). Toxicity was evaluated using electroretinography (b-wave amplitude ratio) for functional outcomes and light microscopy of retinal sections for morphometric analysis 4 weeks after intravitreal injections. The bactericidal efficacy was determined by electroretinography, clinical grading, and histopathologic grading in 15 rabbits, and assessed with bacterial culture of vitreous aspirates in the remaining 15 rabbits.

In the toxicity study, electroretinography waveforms and morphometric results of five intravitreal doses of gatifloxacin were statistically equivalent to rabbits given intravitreal balanced salt solution. In the efficacy study, eyes treated with 200 μg/0.1 mL gatifloxacin and 1000 μg/0.1 mL vancomycin had a significantly better appearance clinically and histologically than untreated control eyes. When compared to antibiotic treated eyes, the untreated control eyes had a significant reduction in b-wave amplitude ratio. Eyes in the
gatifloxacin group showed similar appearance to those in the vancomycin treated group clinically, histologically, and functionally as proved with electroretinography. All aspirates from the gatifloxacin and vancomycin groups were culture-negative 5 days after bacterial inoculation (4 days after therapy), whereas all eyes in the untreated control group were culture-positive. Only one of five eyes in the untreated control group was culture-negative by 7 days after inoculation.

Intravitreal gatifloxacin in doses up to 1000 μg/0.1 mL caused no electroretinographic or retinal histologic abnormality. These results indicate that gatifloxacin is a safe intravitreal antibiotic in rabbit animal model. Furthermore, this study demonstrated that intravitreal injection of 200 μg /0.1mL gatifloxacin seems to be equally effective with intravitreal 1000 μg /0.1 mL vancomycin in the treatment of S. epidermidis endophthalmitis. If proven safe and efficacious by further study in humans, intravitreal injection of gatifloxacin could be considered as an alternative to vancomycin.

Key Words: endophthalmitis, fluoroquinolone, gatifloxacin, intravitreal antibiotics, Staphylococcus epidermidis, vancomycin
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I. INTRODUCTION

Infectious endophthalmitis is an ocular inflammation resulting from the introduction of an infectious agent into the posterior segment of the eye with a intraocular surgery or penetrating ocular injury.\(^1\) The incidence of endophthalmitis after intraocular surgery has been reported to range between 0.05% and 0.37%.\(^2\) The vast majority of confirmed growth isolates (94.2%) were Gram-positive pathogens, most commonly *Staphylococcus epidermidis* (68%).\(^3\) The most common source of infection was patient’s own normal conjunctival and lid flora.\(^4,5\)

Bacterial endophthalmitis is a catastrophic complication that can lead to permanent blindness in a short time if not treated properly and promptly.\(^6\) High
concentrations of antibiotic in the vitreous are required to achieve a bactericidal effect on most major pathogens, and such concentrations are not achievable by systemic and periocular administration. Intravitreal injection of antibiotics has been the most effective method to maintain antibiotic levels inside the eye.\textsuperscript{1,4} When endophthalmitis is initially suspected, the choice of antimicrobial agent must be made empirically. Unfortunately, clinical features of infection and culture results often do not correlate adequately to guide the choice of antibiotics on presentation. Treatment recommendations for endophthalmitis are vancomycin for Gram-positive bacteria and amikacin or ceftazidime for Gram-negative bacteria.\textsuperscript{3} It would be much more beneficial for the patients and more convenient for ophthalmologists if only one antibiotic instead of two could be used to treat both Gram-positive and Gram-negative bacterial endophthalmitis.\textsuperscript{6}

Fluoroquinolone has been widely used in ophthalmology, because of its broad Gram-positive and Gram-negative coverage.\textsuperscript{7-10} Recent reports of ocular bacterial resistance to second- and third-generation fluoroquinolones prompted the introduction of fourth-generation of fluoroquinolone.\textsuperscript{11} Second-generation fluoroquinolone-resistant \textit{Staphylococcus aureus}, coagulase negative staphylococci, and \textit{Streptococcus viridans} have been reported more susceptible to gatifloxacin and moxifloxacin than second- and third-generation fluoroquinolones.\textsuperscript{12} Gatifloxacin is a fourth-generation fluoroquinolone with
broad-spectrum coverage that encompasses organisms commonly encountered in bacterial endophthalmitis.\textsuperscript{13}

Recent report showed that up to 400 $\mu$g/0.1 mL or less of gatifloxacin appeared nontoxic in normal rabbit eyes for 2 weeks without causing any electroretinographic decrease or retinal toxicity on light microscopy.\textsuperscript{14} Histopathologic and electroretinographic studies have demonstrated the retinal safety of intravitreal injection of moxifloxacin, another 4th-generation fluoroquinolone, at a concentration of $\leq 160$ $\mu$g/0.1 mL in the rabbit eye.\textsuperscript{2} Furthermore, a significant reduction in colony counts of \textit{S. epidermidis} in the vitreous was recently shown in a rabbit endophthalmitis model 3 days after intravitreal injection of 50 $\mu$g/0.1 mL of moxifloxacin.\textsuperscript{5} However, there have been no published studies in which intravitreal gatifloxacin has been used for the treatment of experimental \textit{S. epidermidis} endophthalmitis or comparing it with other commonly used drugs.

The goals of this study were twofold. First, it was to evaluate the ocular toxicity of intravitreally administered up to 1000 $\mu$g/0.1 mL of gatifloxacin for 4 weeks using electroretinography (ERG) and light microscopy (LM) in rabbits. Second, it was to compare the efficacy of intravitreal 200 $\mu$g/0.1 mL gatifloxacin, provided it’s safety had been reconfirmed in our toxicity study, with intravitreal 1000 $\mu$g/0.1 mL vancomycin application in the treatment of
experimental *S. epidermidis* endophthalmitis in rabbit eyes.

II. MATERIALS AND METHODS

Fifty-five New Zealand white rabbits weighing 1.5-2 kg were used in this study, in accordance with the guidelines of the Ewha Womans University College of Medicine, Office of Veterinary Affairs. The safety and bactericidal efficacy of gatifloxacin were evaluated. The toxicity of up to 1000 μg/0.1 mL of intravitreal gatifloxacin was investigated using 25 rabbits. The efficacy of intravitreal 200 μg/0.1 mL gatifloxacin was compared with that of intravitreal 1000 μg/0.1 mL vancomycin using ERG, clinical grading, bacterial culture, and histopathology using 30 rabbits. Bacterial culture in 15 rabbits were separately performed from clinical and ERG evaluations because anticipated total 4 sessions of vitreous aspiration might cause more severe inflammation and affect the clinical and ERG recording outcomes.

Gatifloxacin (400 mg, Tequin®, Bristol-Myers Squibb Company, Princeton, NJ, USA) and vancomycin (500 mg, Vancomycin HCl, Dong-A Pharmaceutical Co., Ltd.) were diluted aseptically with sterile balanced salt solution (BSS®, Alcon Korea co., Ltd.) to final concentrations.
1. Toxicity study

Twenty-five rabbits were divided into five equal groups (5 rabbits in each). The right eyes received 0.1 mL intravitreal injections of 1000 μg gatifloxacin in group 1, 500 μg in group 2, 250 μg in group 3, 100 μg in group 4 and 50 μg in group 5. The left eyes served as a control and were injected with 0.1 mL of BSS. Before the injection of gatifloxacin into the vitreous cavity, the rabbits underwent a complete ophthalmic examination including slit lamp biomicroscopy and indirect ophthalmoscopy. All examinations and treatments were performed under general anesthesia with an intramuscular injection of a mixture of xylazine hydrochloride (6 mg/kg) and tiletamine/zolazepam (15 mg/kg). Mydriasis was achieved with topical tropicamide 1% and phenylephrine hydrochloride 2.5% ophthalmic solutions. Under direct examination, all intravitreal injections were made with a 30-gauge needle attached to a 1-mL tuberculin syringe approximately 2 mm from the superonasal limbus positioned in the midvitreous cavity, with the bevel directed away from the retina. The total amount of fluid during injection was 0.1 mL. To maintain normal intraocular pressure, anterior chamber paracentesis was performed prior to intravitreal injection.

ERG using the UTAS-2000 (LKC Technologies, Gaithersburg, MD, USA) was performed before intravitreal injections and 4 weeks after intravitreal injections.
The rabbits were dark adapted for at least 30 minutes after pupillary dilation. Unipolar contact lenses (ERG-jet®, Universo Ltd, La Chaux-de-Fonds, Switzerland) were put on both corneas. The reference electrode (GRD-SAF, The Electrode Store, Leominster, United Kingdom) was inserted into the forehead, and the ground electrode (GRD-SAF, The Electrode Store) was inserted into the ear. Dark adapted maximal combined responses were recorded. The average of three sweeps was determined for each animal. ERG analysis was based on amplitude measurements of the b-wave. The b-wave amplitude was defined from the trough of the a-wave to the peak of the b-wave. Because the ERG responses were recorded simultaneously from the experimental and the control eyes, it was assumed that most factors contributing to the ERG variability similarly affected both eyes. Thus, the b-wave ratio (experimental eye: control eye) was used as an index for retinal function in the experimental eye.15,16

At the end of 4 weeks, the rabbits were killed after ERG tests by putting the animals in the CO₂ gas chamber for 60 seconds. The eyes were enucleated immediately, hemisectioned at the optic nerve, and subsequently were fixed in 10% formalin for at least 24 hours, embedded in paraffin, and sectioned at 2-3 μm. Sections were then stained with hematoxylin and eosin and examined by a pathologist.

For morphometric analysis, mounted sections were microscopically observed
and the measurements were performed with the aid of software program (Analysis®, Soft Imaging System GmbH, Münster, Germany) at the same magnification in four defined retinal locations.\textsuperscript{17-19} in the central retina, at 1 mm from the optic disc (on both sides), and at the equator (on both sides). Numeric values of these four locations were averaged for each eye, and were used in the statistical analysis. To quantify the degree of retinal damage, we measured total retinal thickness of each eye (assessed as distance from the retinal pigment epithelium to the inner limiting membrane; expressed in μm), the thickness of the inner plexiform layers in micrometers (distance from the inner nuclear layer to the ganglion cell layer), and linear cell density (in the retinal ganglion cell layer within 1 mm of the optic disc; expressed as the number of nuclei in a 1-mm-wide band, cells/mm). No attempt was made to distinguish the cell types in the ganglion cell layer for counting the cell number.

Data were expressed as mean ± SD. Statistical calculation was made by using the Kruskal-Wallis test and Wilcoxon test as indicated. A p value less than 0.05 was considered statistically significant (SPSS version 11.0).

2. Efficacy study

\textit{S. epidermidis} (Korean Collection for Type Culture #3958) was used as the challenging organism. Before the experiment, the organism was thawed from
storage at -70°C and plated onto blood agar plate for purity. The strain was incubated for 15-18 hours at 37°C on blood agar plate. Bacteria were harvested by centrifugation, washed and suspended in sterile Hanks’ balanced salt solution (HBSS). The turbidity of the bacterial suspension was adjusted to give 0.15 absorbance at 625 nm wavelength. The bacterial suspension at this turbidity contains approximately 1x 10⁷ colony-forming unit (CFU)/mL. This suspension of *S. epidermidis* was adjusted by serial dilution in sterile saline to obtain a final concentration of approximately 1x 10⁶ CFU/mL for intravitreal inoculation. Colony counts were performed to verify the actual concentration. *S. epidermidis*, the strain used in this study, proved to be susceptible to oxacillin as determined by Sens-Disc™ Susceptibility Test (Dickinson and Company Sparks, Becton, MD, USA).

We diluted the gatifloxacin and vancomycin with Muller-Hinton broth (Difco, Detroit, MI, USA) to obtain minimum inhibitory concentration (MIC) ranging from 0.125 to 512 μg/mL. An inoculum of the *S. epidermidis*, the strain used in this study, was 5 x 10⁵ CFU/mL in each test tube. The lowest antimicrobial concentration that completely inhibit visible bacterial growth was recorded as the MIC. The MIC of gatifloxacin and vancomycin for *S. epidermidis* were 0.25 μg/mL and 4 μg/mL, respectively.

Fifteen rabbits were divided into 3 groups. Each group consisted of 5 rabbits.
The rabbits were prepared for injection as in toxicity study. On day 1, a total of 0.1 mL of the bacterial suspension (10^5 CFU *S. epidermidis*) was injected into the midvitreous cavity of the right eye of each rabbit via the pars plana, approximately 2 mm posterior to the limbus by using 30-gauge needle attached to a tuberculin syringe. Immediately prior to the inoculation of bacteria, anterior chamber paracentesis was performed to limit the intraocular pressure increase and to avoid extrusion of the inoculum.

ERGs were performed as in toxicity study before the injection of *S. epidermidis* and 7 days after bacterial inoculation (6 days after antibiotics and BSS injection).

After confirming the presence of clinical signs of endophthalmitis such as moderate to severe conjunctival injection with vitreous haze which at least partially obscures retinal vasculature, the eyes randomly assigned to one of the three groups equally. Twenty-four hours after intravitreal inoculation of *S. epidermidis*, the right eyes of the rabbits in group 1 received 200 μg/0.1 mL gatifloxacin. In group 2, the right eyes of rabbits received 1000 μg/0.1 mL vancomycin. Group 3 eyes received 0.1 mL BSS and served as untreated infected controls. The left eyes served as uninfected normal controls.

The eyes of rabbits in each group were examined on the 1st, 2nd, 3rd, and 6th day after the intravitreal injection of *S. epidermidis* using slit lamp
biomicroscopy and indirect ophthalmoscopy. The degree of inflammation was quantified by using a standardized grading scheme, which attributes a numerical score to clinical findings\textsuperscript{20} (Table 1). The individual score for the anterior chamber and iris was multiplied by a factor of 2, and the vitreous and retina findings were multiplied by a factor 3.

Table 1. Grading scheme for clinical observation

<table>
<thead>
<tr>
<th>Score</th>
<th>Cornea</th>
<th>Iris and anterior chamber</th>
<th>Vitreous and retina</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal appearance</td>
<td>Normal appearance</td>
<td>Normal appearance</td>
</tr>
<tr>
<td>1</td>
<td>Mild stromal haze</td>
<td>Mild hyperemia, cells in anterior chamber</td>
<td>White condensation in vitreous, petechial hemorrhages along nerve-vessel-streaks</td>
</tr>
<tr>
<td>2</td>
<td>Moderate stromal haze</td>
<td>Moderate hyperemia, exudate in anterior chamber</td>
<td>Vitreous haze, optic disc and large vessels still visible</td>
</tr>
<tr>
<td>3</td>
<td>Cornea opaque</td>
<td>Iris vessels engorged and massive exudate in anterior chamber, or iris not visualizable because of anterior chamber inflammation</td>
<td>No red reflex</td>
</tr>
</tbody>
</table>

13
The total score was calculated by the sum of points from the cornea, iris and anterior chamber, and vitreous and retina. The final score was determined by dividing the total score by 6, resulting in a number ranging from 0 (normal appearance) to 3 (severe inflammatory changes).

The rabbits were killed after the last ERG testing on the 7th day after bacterial inoculation. By 26-gauge needle attached to a 1-mL syringe, 0.1 mL of vitreous aspirates was obtained for microbiological analysis just before the eyes were enucleated. Cultures were considered negative if they did not show growth within 48 h at 37°C. The eyes were enucleated, and prepared for histologic examination as described in the toxicity study.

Histopathologic grading scheme was used to quantify the degree of inflammation of the ocular structures\(^\text{20}\) (Table 2). Cornea, ciliary body, vitreous base, and retina were assigned points according to the described features. To emphasize the importance of the findings in the posterior segment, vitreous points were multiplied by 2 and retinal points by 3. Points were added up, and the mean total score calculated. The total score was then divided by 7 to give a range from 1 (no pathologic changes) to 5 (severe inflammatory changes).
Table 2. Grading scheme for histopathologic evaluation

<table>
<thead>
<tr>
<th>Score</th>
<th>Cornea and limbus</th>
<th>Ciliary body</th>
<th>Vitreous base</th>
<th>Retina</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>2</td>
<td>0-1</td>
<td>0-50</td>
<td>0-20</td>
<td>Cystoid changes, thickness increased</td>
</tr>
<tr>
<td></td>
<td>Inflammatory cells in cornea, mild limbal infiltrate</td>
<td>Inflammatory cells per 40x field</td>
<td>Inflammatory cells per 40x field</td>
<td>up to twice normal, few infiltrates</td>
</tr>
<tr>
<td>3</td>
<td>1-3 Corneal infiltrates, marked limbal infiltrates</td>
<td>50-100</td>
<td>20-50</td>
<td>Thickness increased to more than twice normal, mild infiltrate, photoreceptors recognizable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inflammatory cells per 40x field</td>
<td>Inflammatory cells per 40x field</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3-5 Infiltrates, marked limbal infiltrates</td>
<td>100-200</td>
<td>50-150</td>
<td>Retinal layers discernible, marked leukocytic infiltrates, no photoreceptors recognizable</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inflammatory cells per 40x field</td>
<td>Inflammatory cells per 40x field</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>3-5 Infiltrates, severe limbal infiltrates and swelling</td>
<td>&gt;200</td>
<td>&gt;150</td>
<td>No discernible retinal layers, massive inflammatory infiltrate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inflammatory cells per 40x field</td>
<td>Inflammatory cells per 40x field</td>
<td></td>
</tr>
</tbody>
</table>

Data were expressed as mean ± SD. Statistical calculation was made by using the Kruskal-Wallis test and Wilcoxon test as indicated. A p value less than
0.05 was considered statistically significant (SPSS version 11.0).

The remaining fifteen rabbits were equally divided into three groups (n = 5). All of the rabbits were infected by inoculation of $10^5$ CFU of *S. epidermidis* to the right eyes. Twenty-four hours after the inoculation of bacteria, group 4 received intravitreal 200 μg/0.1 mL gatifloxacin, group 5 received a 1000 μg/0.1 mL vancomycin. Group 6 received 0.1 mL of BSS and was served as untreated infected control. The left eye of each animal was used as an uninoculated control eye.

After 36 h, 3 days, 5 days, and 7 days of the inoculation of *S. epidermidis*, the rabbits were anesthetized as described above. By 23-gauge needle attached to a 2-mL syringe, 0.2 mL of vitreous was aspirated from the midvitreous cavity through the pars plana. After the last aspiration of the vitreous, the rabbits were killed, and their eyes were enucleated, and prepared for histologic examination as described.

Histopathologic grading scheme was used to quantify the degree of inflammation of the ocular structures (Table 2). Samples of vitreous were serially diluted and plated for quantification on blood agar and incubated at 37°C for 48 h. After the incubation period, colonies were counted and identified as *S. epidermidis*.

Results were expressed as mean ± SD. Differences among groups were
tested for significance using a Kruskal-Wallis test. Mann-Whitney U test was used for comparison of two groups. Chi-Square test and Wilcoxon test were used as indicated. A p value less than 0.05 was considered statistically significant (SPSS version 11.0).

III. RESULTS

1. Toxicity study

The b-wave amplitude ratio was calculated by dividing the amplitude of the b-wave measured in the right eye by the corresponding value recorded from the left eye. There were no significant differences for baseline ERGs and ERGs recorded 4 weeks after various doses of gatifloxacin injections among all groups (Table 3).

ERG recordings of eyes from group 1 (gatifloxacin 1000 μg/0.1 mL) revealed that there were no significant changes in b-wave amplitude ratios between before and after gatifloxacin injection (Figure 1).

Histopathologic examination of the retinas by light microscopy revealed normal findings in all groups. The inner and outer layers of the retinas showed no signs of cytotoxicity or disorganization of the cytoarchitecture when compared with those of control retinas. The photoreceptor population density
Table 3. Average ERG ratio\(^1\) with various intravitreal concentrations of gatifloxacin

<table>
<thead>
<tr>
<th>Dose ((\mu)g/0.1 mL)</th>
<th>Preinjection (^*)</th>
<th>4 weeks after injection (^†)</th>
<th>(p) value (^‡)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>1.19 ± 0.19</td>
<td>1.14 ± 0.19</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>5000</td>
<td>0.97 ± 0.11</td>
<td>0.96 ± 0.10</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>250</td>
<td>1.01 ± 0.13</td>
<td>0.98 ± 0.12</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>100</td>
<td>0.93 ± 0.17</td>
<td>1.04 ± 0.05</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>50</td>
<td>0.95 ± 0.11</td>
<td>1.16 ± 0.12</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

\(^1\) b-wave amplitude ratio of eyes injected with gatifloxacin compared with contralateral control eyes.

\(^*\) There were no statistically significant differences among all groups (\(p = 0.121\), Kruskal-Wallis test).

\(^†\) There were no statistically significant differences among all groups (\(p = 0.129\), Kruskal-Wallis test).

\(^‡\) Wilcoxon signed ranks test.

appeared the same in all eyes (Figure 1 A, B). Morphometric results for each group are shown in table 4. Quantification of the effects of various dosage of gatifloxacin revealed no obvious changes in linear cell density of the retinal ganglion cell layer, thickness of the inner plexiform layer, and total retinal thickness in experimental groups when compared with those in controls.
Figure 1. ERG waveforms obtained from both eyes of one rabbit in group 1 before (A) and 4 weeks after (B) gatifloxacin 1000 μg/0.1 mL intravitreal injection. Upper curves (wave 1) correspond to ERG of the experimental eye and lower curves (wave 2) represent ERG from contralateral normal eye in each ERG graph. There is no manifest difference in b-wave amplitude ratios (experimental/contralateral) between pre-and postinjection. Short arrows, trough of a-waves; long arrows, peak of b-waves.
Table 4. Morphometric analysis of the rabbit retinas after intravitreal injection of various doses\(^1\) of gatifloxacin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1000 µg</th>
<th>500 µg</th>
<th>250 µg</th>
<th>100 µg</th>
<th>50 µg</th>
<th>Control</th>
<th>p *</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCD, RGC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>27.8 ± 20.9</td>
<td>48.8 ± 5.3</td>
<td>51.6 ± 5.1</td>
<td>55.2 ± 7.5</td>
<td>52.8 ± 7.7</td>
<td>49.42 ± 6.6</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>Total RT, µm</td>
<td>147 ± 2.9</td>
<td>150 ± 5.0</td>
<td>152.2 ± 6.1</td>
<td>151.2 ± 7.9</td>
<td>149.5 ± 4.2</td>
<td>150.6 ± 6.5</td>
<td>&gt;.05</td>
</tr>
<tr>
<td>IPL, µm</td>
<td>32 ± 4.0</td>
<td>38.2 ± 5.8</td>
<td>36.8 ± 3.4</td>
<td>33.4 ± 5.3</td>
<td>35.5 ± 10.9</td>
<td>32.9 ± 7.5</td>
<td>&gt;.05</td>
</tr>
</tbody>
</table>

\(^1\) in 0.1 mL of BSS.

* Kruskal-Wallis test.

LCD, Linear cell density; RGC, Retinal ganglion cell/mm; RT, Retinal thickness; IPL, Inner plexiform layer. Values are means ± SD.

Figure 2. Histopathologic sections of retina by light microscopy in a study eye injected with 1000 µg/0.1 mL of gatifloxacin in group 1 (B), and control eye (A). There is no manifest difference in histopathologic evaluation between the eyes (× 200, H & E). GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; ONL, outer nuclear layer.
2. Efficacy study.

The clinical scores showed no significant difference among all groups on the first day of bacterial inoculation (p = 0.33). Gatifloxacin and vancomycin treated groups had significantly lower clinical scores than untreated infected group 2, 3, and 6 days after inoculation (p = 0.035, 0.012, 0.014, respectively).

Table 5. Statistical summary of the clinical scores and histopathologic results

<table>
<thead>
<tr>
<th>Group</th>
<th>Clinical scores(^1) at</th>
<th>Histopathologic scores(^\Pi)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 2(^*)</td>
<td>day 3(^†)</td>
</tr>
<tr>
<td>Gatifloxacin (200 μg/0.1 mL)</td>
<td>0.95 ± 0.23</td>
<td>1.53 ± 0.44</td>
</tr>
<tr>
<td>Vancomycin (1 000 μg/0.1 mL)</td>
<td>1.13 ± 0.27</td>
<td>1.37 ± 0.42</td>
</tr>
<tr>
<td>Infected control (BSS)</td>
<td>1.25 ± 0.29</td>
<td>2.04 ± 0.09</td>
</tr>
</tbody>
</table>

\(^1\) Mean clinical scores are calculated on day 2, 3, 4, and 7 which corresponds to 1, 2, 3, and 6 days after bacterial inoculation, respectively.

\(^*\) The difference among groups is not statistically significant (p = 0.33, Kruskal-Wallis Test).

\(^†\) The difference among groups is statistically significant (p = 0.035, Kruskal-Wallis Test).

\(^‡\) The difference among groups is statistically significant (p = 0.012, Kruskal-Wallis Test).

\(^§\) The difference among groups is statistically significant (p = 0.014, Kruskal-Wallis Test).

\(^\Pi\) The difference among groups is statistically significant (p = 0.020, Kruskal-Wallis Test).
There were no significant differences in clinical scores between gatifloxacin and vancomycin groups throughout the experiment (Table 5).

ERG b-wave amplitude ratio was not reduced 7 days after inoculation (6 days after antibiotics injection) compared to preinoculation in gatifloxacin and vancomycin treated groups (p = 0.116, 0.498, respectively). In the untreated infected group, b-wave amplitude ratio much decreased 7 days after inoculation compared to preinoculation, but the difference was not statistically significant (p = 0.068). When compared to treatment groups, the untreated infected group had a significant reduction in b-wave amplitude ratio (p = 0.008) (Table 6).

The b-wave implicit time ratio significantly increased 7 days after inoculation compared to preinoculation in the gatifloxacin and vancomycin injected groups (p = 0.028, 0.043, respectively) (Table 7). This suggests that there might be some functional damage in retina due to infection in spite of intravitreal antibiotic administration.
Table 6. Average ERG ratio\(^1\) of each study group

<table>
<thead>
<tr>
<th>Group</th>
<th>Preinoculation*</th>
<th>7 days after inoculation(^\dagger)</th>
<th>p(^\ddagger)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gatifloxacin (200 μg/0.1 mL)</td>
<td>1.09 ± 0.17</td>
<td>0.98 ± 0.13</td>
<td>0.116</td>
</tr>
<tr>
<td>Vancomycin (1000 μg/0.1 mL)</td>
<td>0.99 ± 0.13</td>
<td>0.90 ± 0.36</td>
<td>0.498</td>
</tr>
<tr>
<td>Infected control (BSS 0.1 mL)</td>
<td>1.07 ± 0.11</td>
<td>0.29 ± 0.15</td>
<td>0.068</td>
</tr>
</tbody>
</table>

\(^1\) b-wave amplitude ratio of eyes inoculated with *S. epidermidis* compared with contralateral control eyes.

* There were no statistically significant differences among all groups (p = 0.628, Kruskal-Wallis test).

\(^\dagger\) There were statistically significant differences among all groups (p = 0.008, Kruskal-Wallis test).

\(^\ddagger\) Wilcoxon signed ranks test.

Table 7. Average ERG ratio\(^1\) of each study group

<table>
<thead>
<tr>
<th>Group</th>
<th>Preinoculation*</th>
<th>7 days after inoculation(^\dagger)</th>
<th>p(^\ddagger)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gatifloxacin (200 μg/0.1 mL)</td>
<td>1.01 ± 0.11</td>
<td>1.45 ± 0.15</td>
<td>0.028</td>
</tr>
<tr>
<td>Vancomycin (1000 μg/0.1 mL)</td>
<td>1.08 ± 0.04</td>
<td>1.34 ± 0.10</td>
<td>0.043</td>
</tr>
<tr>
<td>Infected control (BSS 0.1 mL)</td>
<td>1.07 ± 0.12</td>
<td>1.34 ± 0.30</td>
<td>0.114</td>
</tr>
</tbody>
</table>

\(^1\) b-wave implicit time ratio of eyes inoculated with *S. epidermidis* compared with contralateral control eyes.

* There were no statistically significant differences among groups (p = 0.297, Kruskal-Wallis test).

\(^\dagger\) There were no statistically significant differences among all groups (p = 0.543, Kruskal-Wallis test).

\(^\ddagger\) Wilcoxon signed ranks test.
ERG recordings in treated groups showed similar b-wave amplitude to preinoculation but extended b-wave implicit time on the 7th day of bacterial inoculation (Figure 3, A and B). The ERG waves were nearly extinguished in the untreated infected group 7 days after inoculation (Figure 3, C).

All vitreous aspirates from the three groups 7 days after bacterial inoculation were negative for bacterial growth.

The mean histopathologic scores were $1.78 \pm 0.40$, $1.90 \pm 0.28$, and $3.04 \pm 0.52$ in gatifloxacin, vancomycin, and infected control groups, respectively (Table 5). The difference among groups was statistically significant ($p = 0.020$, Kruskal-Wallis test). The untreated infected group had significantly higher scores compared to gatifloxacin and vancomycin treated groups. There was no significant difference between the gatifloxacin and vancomycin treated groups. More severe inflammatory changes in the retina, ciliary body, and vitreous were shown in the untreated infected group than in the gatifloxacin and vancomycin treated groups (Figure 4).
Figure 3. ERG responses obtained from eyes of groups 1 (A), 2 (B), and 3 (C). In each graph, the upper curves (wave 1) correspond to the ERG of experimental eyes and lower curves (wave 2) represent ERG from contralateral normal eyes. Baseline ERG before inoculation of *S. epidermidis* is on the left and the ERG of the same eyes 7 days after bacterial inoculation (6th day of antibiotics injection) is on the right. In gatifloxacin (A) and vancomycin (B) administered groups, the b-wave amplitude ratios (experimental/contralateral) are not changed, but b-wave implicit time ratios (experimental/contralateral) increased 7 days after bacterial inoculation. In infected control group (C), the wave of experimental eye is nearly extinguished. Short arrows, trough of a-waves; long arrows, peak of b-waves.
Figure 4. Histopathologic features of retina, ciliary body, and vitreous in three eyes from each group (H & E). Gatifloxacin (A) and vancomycin (B) treated eyes shows relatively clear vitreous and well preserved retinal layers. In infected control group (C), retina, ciliary body, and vitreous were infiltrated with many inflammatory cells. Left column shows retina, choroid, and sclera. x200 (A and B), x100 (C). Right column represents ciliary body and vitreous base. x40 (A, B, and C). V, vitreous cavity; R, retina; C, choroids; S, sclera; CB, ciliary body.
The differences of mean colony counts of vitreous aspirates among groups after 36 h, 3, 5, and 7 days of bacterial inoculation were statistically significant (p = 0.006, 0.005, 0.001, and 0.007, respectively, Kruscal-Wallis test). Untreated infected group had significantly higher number of CFU compared to gatifloxacin (p = 0.008, 0.008, 0.008, and 0.032, respectively, Mann-Whitney test) and vancomycin (p = 0.008, 0.008, 0.008, and 0.032, respectively, Mann-Whitney test) groups. There was no difference between the gatifloxacin and vancomycin treated groups (p = 0.222, 0.548, 1.00, and 1.00, respectively, Mann-Whitney test) (Table 8).

Bacterial viability was also expressed as culture positivity and statistical calculation was performed by using the Chi-Square test (Table 9). After 36 h of inoculation, four of 5 eyes in gatifloxacin group, three of 5 eyes in vancomycin group, and all eyes in the untreated infected group were culture positive (p = 0.725). On the 3rd day of inoculation, two of 5 eyes in gatifloxacin group, one of 5 eyes in vancomycin group, and all eyes in the untreated infected group were culture positive (p = 0.068). At the 5th and 7th day of inoculation, all eyes in the treatment groups were culture-negative, but five and four eyes still were culture positive in the untreated infected group. Besides, those differences were statistically significant among groups (p = 0.001, 0.011, respectively). Overall, both gatifloxacin and vancomycin treatments succeeded
Table 8. Culture results

<table>
<thead>
<tr>
<th>Group</th>
<th>CFU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 3*</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>6586 ± 10521.11</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>65 ± 92.33</td>
</tr>
<tr>
<td>Infected control</td>
<td>1.25x10^7 ± 1.04x10^7</td>
</tr>
</tbody>
</table>

Day 3, 4, 6, and 8 corresponds to 36 h, 3, 5, and 7 days after inoculation, respectively.

* After 36 h, 3, 5, and 7 days of inoculation, vitreous aspirates were obtained and inoculated on the blood agar plate and incubated for 48 h at 37°C. After the incubation period, surface colonies were counted and converted into CFU/mL.

† The difference among groups is statistically significant (p = 0.005, Kruskal-Wallis Test).

‡ The difference among groups is statistically significant (p = 0.001, Kruskal-Wallis Test).

§ The difference among groups is statistically significant (p = 0.007, Kruskal-Wallis Test).

Gatifloxacin, 200 μg/0.1 mL; Vancomycin 1000 μg/0.1 mL; Infected control, BSS 0.1 mL.

in sterilizing the eyes within 5 days after inoculation (four days after initiation of therapy), but only one eye was autoclaved in the untreated infected group by 7 days after inoculation. There were no significant differences in number of
eyes showed culture positive between the gatifloxacin and vancomycin treated groups after 36 h, 3, 5, and 7 days of inoculation.

The mean histopathologic scores were 2.02 ± 0.39, 2.04 ± 0.39, and 4.14 ± 0.57 in gatifloxacin, vancomycin, and infected control groups, respectively. There was no significant difference between gatifloxacin and vancomycin treated groups, but untreated infected group had significantly higher scores compared to antibiotic treated groups (p = 0.011, Kruskal-Wallis Test). The histopathologic results supported the findings obtained by culture study and clinical observation study. A moderate inflammation of ciliary body and vitreous was shown in the treated groups (Figure 5, A and B), but more severe inflammatory changes were observed in the untreated infected group (Figure 5, C). Overall inflammatory cell infiltration in ocular structures were more severe in the bacterial culture study than in the clinical observation study (Figure 4).
Table 9. Culture results expressed as culture positivity\(^1\)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of eyes that shows culture positive after 48 h of incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>day 3(^*)</td>
</tr>
<tr>
<td>Gatifloxacin (200 μg/0.1 mL)</td>
<td>N=5</td>
</tr>
<tr>
<td></td>
<td>4 (80%)</td>
</tr>
<tr>
<td>Vancomycin (1000 μg/0.1 mL)</td>
<td>N = 5</td>
</tr>
<tr>
<td></td>
<td>3 (60%)</td>
</tr>
<tr>
<td>Infected control (BSS 0.1 mL)</td>
<td>N = 5</td>
</tr>
<tr>
<td></td>
<td>5 (100%)</td>
</tr>
</tbody>
</table>

Day 3, 4, 6, and 8 corresponds to 36 h, 3, 5, and 7 days after inoculation, respectively.

\(^1\) After 36 h, 3, 5, and 7 days of inoculation, vitreous aspirates were obtained and inoculated on the blood agar plate and incubated for 48 h at 37°C. After the incubation period, number of eyes that show culture positive are counted.

\(^*\) The difference among groups is not statistically significant (p = 0.725 Chi-Square Test).

\(^†\) The difference among groups is not statistically significant (p = 0.068, Chi-Square Test).

\(^‡\) The difference among groups is statistically significant (p = 0.001, Chi-Square Test).

\(^§\) The difference among groups is statistically significant (p = 0.011, Chi-Square Test).
Figure 5. Histopathologic features of retina, ciliary body, and vitreous in group 4 (A), 5 (B), and 6 (C) (H & E). Gatifloxacin (A) and vancomycin (B) treated eyes show relatively clear vitreous and well preserved retinal layers. Some inflammatory cells are observed in the vitreous cavity. In untreated infected control group (C), retina, ciliary body, and vitreous were infiltrated with dense inflammatory cells and no retinal layer is intact. Left column shows retina, choroids, and sclera. x200 (A and B), x100 (C). Right column represents ciliary body and vitreous base. x40 (A, B, and C). V, vitreous cavity; R, retina; C, choroids; S, sclera; CB, ciliary body.
V. DISCUSSION

Endophthalmitis is a serious complication of ocular surgery that causes blindness in about half of eyes despite early treatment. In order to cover a broad spectrum of pathogens in initial therapy without specific knowledge of the causative pathogen, it is advisable to use highly potent antibiotics with activity against both Gram-positive and Gram-negative bacteria.\textsuperscript{3,18}

Intravitreal antibiotic injection seems to be the superior regimen recommended for the treatment of endophthalmitis, because the intraocular penetration of systemic antibiotics is poor and intraocular concentration of antibiotics after intravitreal injection is far greater than that achieved by any other modality.\textsuperscript{21-23}

Although vancomycin is most commonly used antibiotics for Gram-positive pathogen coverage at present, there has been increasing evidence of resistance to it.\textsuperscript{24,25}

The fourth generation fluoroquinolones (gatifloxacin and moxifloxacin) have potency equal to the second- and third-generation drugs against Gram-negative bacteria and are more potent than the second- and third-generation fluoroquinolones against Gram-positive bacteria.\textsuperscript{6} Low minimum inhibitory concentration (MIC) and higher tissue concentrations are required for effective activity against antibiotic-resistant bacteria.\textsuperscript{2} Unlike the second- and third-
generation fluoroquinolones, which bind to only one of the two key enzymes involved in bacterial DNA replication, gatifloxacin binds both enzymes: bacterial DNA gyrase and topoisomerase IV. More extensive binding provides more effective bacterial killing and less bacterial mutation into resistant pathogens. It has been demonstrated that gatifloxacin’s MICs are much lower than those of the older fluoroquinolones by one fourth to one eighth for Gram-positive bacteria. Gatifloxacin is being used topically with great success in the treatment of severe corneal and conjunctival infections. In humans, orally administered gatifloxacin was reported to achieve an inhibitory concentration in the aqueous for most of the frequently isolated pathogens responsible for the endophthalmitis. It was concluded that oral gatifloxacin could be considered in the prophylaxis and adjunctive therapy of bacterial endophthalmitis. Topical application of gatifloxacin was shown to reach sufficient intraocular levels to prevent endophthalmitis in an animal model and in humans and surgical prophylaxis with topical gatifloxacin was suggested to be a valuable adjunct for the prevention of endophthalmitis.

Any new antibiotic therapy must be relatively nontoxic to the patient, effective in eliminating infection, and be at least comparable or better than standard therapy. This experimental study was designed to examine whether gatifloxacin can be safely used as an intravitreal agent in the treatment of S.
*S. epidermidis* endophthalmitis in rabbit model.

Intravitreal gatifloxacin did not have retinal toxicity in normal rabbit eyes in doses of 1000 μg/0.1 mL or less. The results of our study concur with the findings of Kazi et al.,¹⁴ who also studied the intravitreal toxicity of gatifloxacin. They injected a maximum concentration of 400 μg/0.1 mL of intravitreal gatifloxacin, whereas we injected a dose up to 1000 μg/0.1 mL. They did not find any retinal toxicity on ERG and light microscopy two weeks after intravitreal injection, similarly there was no retinal toxicity on ERG and light microscopy 4 weeks after intravitreal injection in our study.

It has been suggested that effective treatment of endophthalmitis requires a 2- to 10-fold increase over the MIC₉₀ (the concentration of drug causing a 90% growth inhibition of organisms) for most of the causative organisms.¹² In the inflamed and infected eye, the mechanism of active transport across the retina is compromised, and drugs such as fluoroquinolones that are cleared via the posterior route have an increased half-life in the vitreous.³⁴ Therefore, maximum nontoxic dose of gatifloxacin in normal eyes may have some toxicity in the already inflamed eye due to an infection. *S. epidermidis* has relatively low virulence and causes slow progression. The MIC₉₀ of gatifloxacin for *S. epidermidis* is in the range of 0.09 to 2.0 μg/mL.¹² Moreover, moxifloxacin, another 4th generation fluoroquinolone, has been proved more potent than
gatifloxacin and has significantly decreased (not eliminated) the bacteria in the vitreous 3 days after intravitreal injection with a dose of 50 μg/0.1 mL in rabbits with experimental *S. epidermidis* endophthalmitis.⁵ Therefore, we had decided the trial dose of gatifloxacin for the treatment of endophthalmitis as 200 μg/0.1 mL beforehand, because it is between 50 to 400 μg/0.1 mL and is much higher than the required effective dose of drug against the *S. epidermidis* (62 to 1388-fold the MIC₉₀). As the safety of intravitreal 200 μg/0.1 mL gatifloxacin had been proved again in our toxicity study, we used gatifloxacin at a dose of 200 μg/0.1 mL for following efficacy study.

Our results proved that both intravitreal 200 μg/0.1 mL gatifloxacin and 1000 μg/0.1 mL vancomycin successfully sterilized infected eyes in a rabbit model of *S. epidermidis* endophthalmitis and significantly ameliorate anterior and posterior segment inflammation compared to untreated infected eyes. Electrophysiological, microbiological and histopathologic results confirmed the clinical findings. In the efficacy study for bacterial culture, all eyes in gatifloxacin and vancomycin treated groups had no growth according to the culture results of vitreous samples within 5 days after inoculation whereas untreated infected controls demonstrated 100% and 80% of culture positivity after 5 and 7 days of inoculation, respectively. In the efficacy study for clinical examination and ERG recording, vitreous aspiration for bacterial culture was
performed once immediately before enucleation (7 days after bacterial inoculation) and all eyes showed no growth irrespective of treatment. These results suggested that autosterilization of *S. epidermidis* could occur in rabbits with endophthalmitis. Previous reports concluded that the number of colony, virulence of bacteria, the variability in bacterial strains, and the state of ocular condition (aphakia, pseudophakia, vitrectomized) might affect the autosterilization with experimental endophthalmitis. Only one of five eyes in the untreated infected group was autosterilized 7 days after inoculation in the efficacy study for bacterial culture. Several sessions of vitreous tapping might cause more severe inflammation and assist the bacteria to replicate. Mean histopathologic scores of each group in the bacterial culture study were higher than those of corresponding group in the clinical observation study. Moreover, untreated infected eyes in the bacterial culture study showed the most severe ocular destruction.

Based on the study by Mather et al., gatifloxacin has an in vitro MIC$_{90}$ of $\leq$ 3.5 $\mu$g/mL for all of the bacterial endophthalmitis isolates, including the second-generation fluoroquinolones-resistant staphylococci. The vitreous volume of rabbit eye is 1.5 mL; injection of gatifloxacin at a concentration of 200 $\mu$g/0.1 mL into the vitreous can therefore produce concentration of 125 $\mu$g/mL. This dose is well above the MIC$_{90}$ values of ocular pathogens that cause
endophthalmitis (35 to 2083-fold).

In recent study on the MICs and susceptibility patterns of bacterial endophthalmitis isolates to fluoroquinolones, the susceptibilities to gatifloxacin of second-generation fluoroquinolone-resistant *S. aureus* and *S. epidermidis* are 12.5% and 60%, respectively.\(^{12}\) Among Gram-positive organisms causing endophthalmitis, resistance to vancomycin is rare. In a study of 8500 strains of bacteria, 97.9% of the Gram-positive organisms were susceptible to vancomycin.\(^{38}\) Recent study demonstrated vancomycin retains in vitro efficacy against more than 99% of Gram-positive bacteria causing endophthalmitis.\(^{39}\) However, the emergence of vancomycin-resistant enterococci causing endophthalmitis is a rising concern.\(^{4,38,40}\) Gatifloxacin could be considered an alternative to vancomycin in selected patients with resistant bacterial infection or allergy to the more traditional antibiotics.

The present study also indicated that the 200 \(\mu g/0.1\ mL\) gatifloxacin offers no advantages over 1000 \(\mu g/0.1\ mL\) vancomycin in the treatment of *S. epidermidis* endophthalmitis. For a more effective treatment, higher dose of gatifloxacin may be needed. Also, the efficacy of gatifloxacin for the treatment of Gram-negative bacteria should be compared with ceftazidime, the standard antibiotic for Gram-negative organisms. The MIC\(_{90}\) of gatifloxacin for *P. aeruginosa*, extremely virulent and destructive Gram-negative organism, is in the range of
0.06 to 32 μg/mL,\textsuperscript{12,31,41} increase of the dosage of gatifloxacin could be used because up to 1000 μg/0.1 mL gatifloxacin was demonstrated nontoxic in our study. Thus, if proven safe and efficacious by further study, the one antibiotic therapy for intravitreal injection in the treatment of endophthalmitis would be possible.

To our knowledge, this is the first report to prove and compare the efficacy of 4th generation fluoroquinolone with vancomycin in the treatment of \textit{S. epidermidis} endophthalmitis. In order to use this agent effectively and safely in the treatment of infectious endophthalmitis, further ocular pharmacokinetic study, dose-response relationship research, and comparison with ceftazidime in the Gram-negative bacterial endophthalmitis are necessary.
V. CONCLUSION

This experimental study was designed to examine the retinal toxicity of intravitreal gatifloxacin using ERG and histology in rabbit model, and to compare the efficacy of intravitreal gatifloxacin with that of vancomycin in rabbit eyes with experimental *S. epidermidis* endophthalmitis using ERG, clinical grading, histology, and bacterial culture.

The results of this study showed that up to 1000 μg/0.1 mL gatifloxacin can be safely tolerated in the normal rabbit eyes for 4 weeks without causing any ERG abnormality or histologic changes on light microscopy.

The present study also demonstrated that intravitreal injection of 200 μg/0.1 mL gatifloxacin is equally effective with intravitreal 1000 μg/0.1 mL vancomycin application in the treatment of *S. epidermidis* endophthalmitis. Therefore, intravitreal gatifloxacin may be an alternative antibiotic in the treatment of *S. epidermidis* endophthalmitis. If future studies in other species confirm our findings, intravitreal gatifloxacin may be a potentially important drug in the treatment and prevention of clinical bacterial endophthalmitis.

To evaluate whether gatifloxacin is more effective in the treatment of endophthalmitis, increase of the dosage of gatifloxacin may be needed. Also, the efficacy of gatifloxacin for the treatment of Gram-negative bacteria, such as *P.*
*aeruginosa* should be compared with that of ceftazidime, the standard antibiotic for Gram-negative pathogens. If proven safe and efficacious by further study, the one antibiotic therapy for intravitreal injection in the treatment of endophthalmitis would be possible.
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실험적 피부포도알균 (Staphylococcus epidermidis) 안내염에서
gatifloxacin과 vancomycin의
유리체강내 주사의 효과

< 지도교수 고 형 준 >
연세대학교 대학원 의학과
이 수 영

토끼에서 gatifloxacin을 유리체강내로 주사했을 때 정상 망막에 독성을 유발하는지 알아보고, 실험적으로 피부포도알균 (Staphylococcus epidermidis) 안내염을 일으킨 뒤 gatifloxacin과 vancomycin의 유리체강내 주사의 효과를 비교해 보고자 하였다.
55마리의 New Zealand white rabbit을 대상으로 독성 연구와 치료 효과 연구를 시행하였다. 독성 연구에서는 25마리의 토끼를 한 군당 5마리씩 다섯 군으로 나누어 다섯 가지 농도의 gatifloxacin (1000, 500, 250, 100, 50 μg/0.1 mL) 을 오른쪽 눈 (실험이 안) 의 유리체강내로 주사하고 왼쪽 눈 (대조안) 에는 balanced salt solution을 유리체강내로 주사하였
으며, 주사 후 4주 뒤에 망막전위도 (b-파 전폭비) 와 광학현미경으로 망막의 기능과 조직학적 변화를 비교하였다. 치료 효과 연구에서는 각각 30마리의 토끼를 한 군당 5마리씩 여섯 군으로 나누어 오른쪽 눈에 실험적으로 피부포도알균 안내염을 유발하였다. S. epidermidis는 10^5 CFU/0.1 mL을 유리체강내로 접종했고, 균접종 후 24시간 뒤에 각 군별로 각기 gatifloxacin 200 μg/0.1 mL (1, 4군), vancomycin 1000 μg/0.1 mL (2, 5군), 그리고 balanced salt solution (3, 6군) 을 유리체강내로 주사하였다. 처음 15마리, 세 군 (1, 2, 그리고 3군) 에서는 망막전위도와 염증검사 그리고 광학현미경상의 조직소견을 이용하여 항생제 치료군과 비치료감염군의 결과를 비교하였다. 나머지 15마리, 세 군 (4, 5, 그리고 6군) 에서는 항생제치료군과 비치료감염군에서의 세균배양검사 결과와 조직검사소견을 비교하였다.

독성 연구 결과, 다섯개 실험군의 망막전위도 결과와 조직학적 소견이 대조군과 비교하여 유의한 차이가 없었다. 치료 효과 연구에서는 gatifloxacin군과 vancomycin군에 비하여 비치료감염군에서 염상검사상 염증정도와 광학현미경 감사상의 조직 손상 정도가 유의하게 심했고, 망막전위도의 b-파 전폭비도 유의하게 감소했다. Gatifloxacin군과 vancomycin 치료군은 염증 정도, 조직 손상 정도, 망막전위도 소견에 차이가 없었다. 균배양검사결과, 항생제 치료군의 경우 균주입 후 5일째 (항생제 주사 후 4일째) 에 채취한 유리체에서 모든 눈 (각기 5안
중 5안) 이 균배양 음성을 보인 반면, 비치료 감염군에서는 모든 눈 (5안 중 5안)이 균배양 양성이었다. 비치료 감염군은 균주입 후 7일 제에도 5안 중 4안이 균배양 양성을 보였다.

본 연구에서 gatifloxacin은 정상 토끼눈에 최고 1000 µg/0.1 mL까지 주사하여도 망막전위도나 조직학적으로 이상소견을 유발하지 않았다. 따라서 gatifloxacin은 토끼에서 유리체강내로 안전하게 주사할 수 있는 항생제라고 할 수 있다. 또한 실험적인 피부포도알균 안내염에서 200 µg/0.1 mL의 gatifloxacin은 1000 µg/0.1 mL의 vancomycin과 동등한 항균효과를 보여주었다. 향후 사람을 대상으로 한 연구에서도 gatifloxacin의 안전성과 효과가 입증된다면 gatifloxacin은 안내염의 치료에 vancomycin을 대신할 수 있는 항생제로 고려할 수 있을 것이다.

핵심되는 말: 세균성안내염, 플루오로퀴놀론, 가티플록사신, 유리체강내 항생제주사, 피부포도알균, 반코마이신