whereas 33% (four out of 12 cases) of FNAB samples obtained with the standard 25G needle were non-diagnostic.

Our pilot study results are based solely upon trans-scleral FNAB approach and the study was not designed to evaluate complication rates. However, encouraged with our preliminary results, a study using trans-vitreal approach is planned. Such a study will also provide comparative data regarding complication rates. Our study results need to be verified independently in a larger number of patients.

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Competing interests None.

Ethics approval Approval provided by the IRB.

Provenance and peer review Not commissioned; internally peer reviewed.

Published Online First 16 May 2012

Br J Ophthalmol 2012;**96**:1147–1148.
doi:10.1136/bjophthalmol-2012-301572

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Expression of prostaglandin F receptor in scleral and subconjunctival tissue

Prostaglandin F2α ($PGF_{2\alpha}$) analogues eye drops are regarded as a first choice for the treatment of glaucoma. The hypotensive action of $PGF_{2\alpha}$ analogues is thought to be attributed to an increase in uveoscleral outflow.[1] However, the underlying mechanisms have yet to be well defined. The purpose of this present study was to examine prostaglandin F receptor (FP) localisation in ocular tissue.

All experiments were conducted in accordance with the principles set forth in the Declaration of Helsinki. The expression of FP was examined by X-gal staining in ocular tissues of FP knockout mice carrying the β-galactosidase gene at the FP loci ($Ptgfr^{-/-}$ mice)[2] and reverse transcription PCR of human conjuncitival and scleral fibroblasts (see supplementary methods, published online only).

First, FP localisation was examined using $Ptgfr^{-/-}$ mice in which the β-galactosidase gene was 'knocked-in' at the FP gene. In the $Ptgfr^{-/-}$ mice, X-gal staining of ocular tissue revealed positive signals in the sclera and subconjunctival tissue, but not the corneal tissue, and dense positive signals in the tarsal muscle of the eyelids (figure 1). The sclera under ciliary body also showed positive signals. However, no positive signals could be found in the corneal or conjunctival epithelium. X-gal staining of the ocular tissue did not reveal positive signals in wild-type mice (data not shown).

Next, reverse transcription PCR analyses were performed. The expected length of PCR products (887 bp) was obtained from cultured human scleral fibroblasts and cultured human conjunctival fibroblasts, but not conjunctival epithelium (figure 2).

We investigated FP localisation in ocular tissue and found that scleral and subconjunctival tissues expressed FP. FP expression of the sclera is consistent with the theory of the increase in uveoscleral outflow.

Previous studies have reported FP localisation in human ocular tissue by in-situ hybridisation[3] or by immunohistochemistry.[4] Using in-situ hybridisation, Mukhopadhyay and associates[3] reported the presence of high levels of FP receptor messenger RNA transcripts in the blood vessels of the iris, choroid and ciliary body, in which FP mRNA transcript was predominantly present in the circular muscle. As C57BL/6 mice have substantial pigment in the uvea, including the iris, choroid and ciliary body, we were unable to detect X-gal staining in these tissues. However, we did

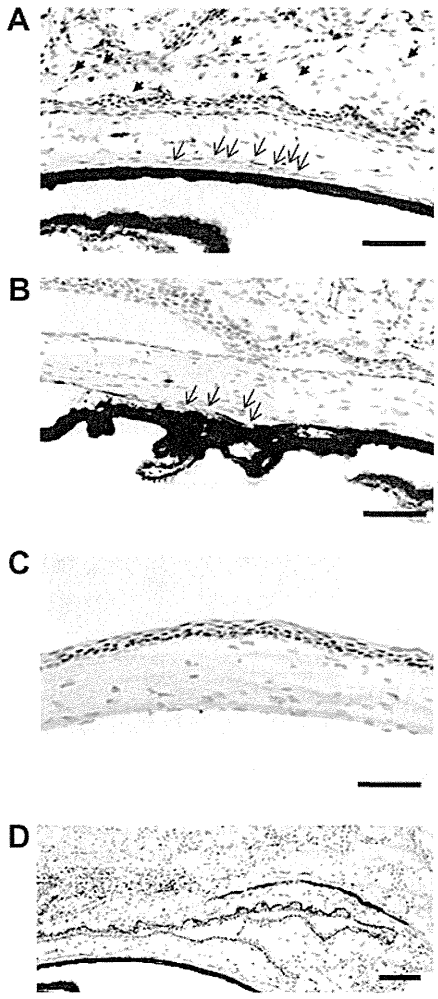

Figure 1 Histochemical staining for prostaglandin F receptor (FP) (X-gal). Ocular tissues from $Ptgfr^{-/-}$ mice expressing the β-galactosidase gene at the *Ptgfr* locus were stained for β-galactosidase activity with the substrate X-gal. Sections of ocular tissues from $Ptgfr^{-/-}$ mice were counterstained with haematoxylin (purple). Positive signals (blue) were shown on conjunctival fibroblasts (arrowhead: A), and sclera (arrow: A and B). (A) The backward sclera and conjunctiva; (B) ciliary body; (C) cornea; (D) tarsal muscle of the eyelid. Data are representative of three experiments (three $Ptgfr^{-/-}$ mice). Each bar represents a length of 100 μm.

detect dense positive signals of X-gal staining in the tarsal muscle of eyelids. FP might possibly be expressed in smooth muscles. On the other hand, we could not find FP expression in the ocular surface epithelium, although the immunohistochemistry findings showed a strong expression of FP in the ocular surface epithelium. We also could not detect FP-specific mRNA in human conjunctival epithelial cells, while we could detect it in human conjunctival and scleral fibroblasts. So, it is true that FP expression in ocular surface epithelial cells is less than in conjunctival and scleral fibroblasts. Furthermore, FP expression in the fibroblast but not

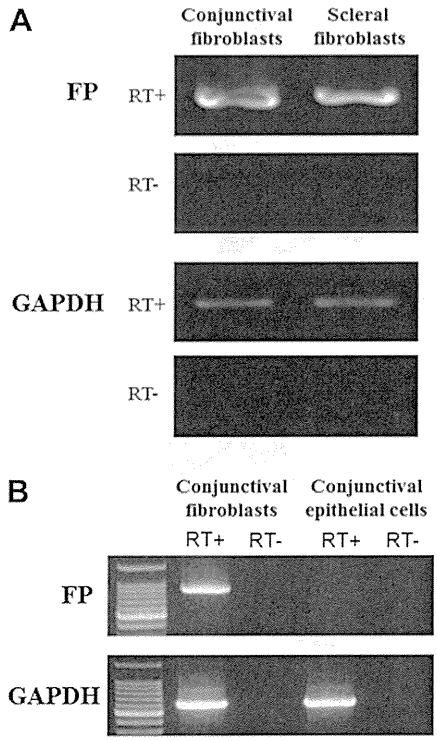

Figure 2 Reverse transcription (RT) PCR analyses of the expression of prostaglandin F receptor (FP)-specific mRNA. (A) Human conjunctival fibroblasts and human scleral fibroblasts; (B) human conjunctival fibroblasts and human conjunctival epithelial cells.

epithelial cells supports the theory of the increase in uveoscleral outflow.

Furthermore, it was also reported that $PGF_{2\alpha}$–FP signalling facilitates pulmonary fibrosis, and that $PGF_{2\alpha}$ could stimulate collagen production of lung fibroblasts via FP independently of transforming growth factor beta.[2] However, in cultured human conjunctival fibroblasts and scleral fibroblasts, transforming growth factor beta, but not $PGF_{2\alpha}$, could facilitate the collagen production in our experiment (data not shown), thus suggesting that $PGF_{2\alpha}$ analogues eye drops might not facilitate the fibrosis on the ocular surface.

In summary, the findings of this study showed that FP, the receptor of $PGF_{2\alpha}$, was expressed not only in the ciliary body, but also in scleral and subconjunctival tissues, suggesting that the $PGF_{2\alpha}$ analogues might affect not only the ciliary muscle but also the sclera and subcojunctiva.

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►An additional material is published online only. To view this file please visit the journal online (http://bjo.bmj.com/content/96/8.toc).

Contributors All authors made material contributions to this research. MU and KI were responsible for writing and reviewing contributions to the manuscript.

Competing interests None.

Ethics approval This study was approved by the Institutional Review Board of Kyoto Prefectural University of Medicine, Kyoto, Japan. All experimental procedures were conducted in accordance with the tenets set forth in the Declaration of Helsinki.

Provenance and peer review Not commissioned; internally peer reviewed.

Published Online First 11 May 2012

Br J Ophthalmol 2012;**96**:1148–1149. doi:10.1136/bjophthalmol-2012-301815

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Endothelial keratoplasty in children: surgical challenges and early outcomes

INTRODUCTION

A significant proportion of paediatric keratoplasties are performed for endothelial dysfunction due to failed graft, congenital hereditary endothelial dystrophy[1] and pseudophakic corneal oedema. Descemet's stripping endothelial keratoplasty (DSEK) is an evolving procedure for isolated endothelial dysfunction with encouraging results in adults. The application and outcome of this procedure in the paediatric population has not been well studied with few reports being published so far.[2–5]

This study reports the indications, surgical technique and early outcomes of DSEK in children <14 years of age.

METHODS

All children who underwent DSEK at our centre between January 2008 and January

Table 1 Preoperative and postoperative clinical data of children undergoing DSEK

Parameters	
Demographics	
Average age (in years)	8.06±3.95 years
Male:Female	3:1
Preoperative visual acuity	
Could not measure	8
<20/400	8
Indications for surgery	
Failed graft (PK performed for congenital hereditary endothelial dystrophy in five, microbial keratitis in two and corneal opacities in two cases; one case had a previous failed DSEK performed elsewhere for endothelial dysfunction following bee sting injury)	10 (62.5%)
Pseudophakic corneal oedema	3 (18.75%)
CHED	2 (12.5%)
Bee sting injury	1 (6.25%)
Surgical intervention	
Endothelial keratoplasty alone	15 (93.75%)
Endothelial keratoplasty + cataract surgery	1 (6.25%)
Follow-up	9.37±5.72 months
Average time for resolution of graft oedema	4.16±1.52 weeks (range 2–8 weeks)
Visual acuity at final follow-up	
Up to counting fingers 3 m	3 (18.75%)
20/400–20/100	8 (50%)
>20/80	5 (31.25%)
Complications	
Cataract in two cases (cataract surgery 2 months after DSEK)	
Glaucoma (Ahmed valve 6 months after DSEK) in one case	
Primary graft failure: one case	
Lenticule detachment in two cases: successful rebubbling in the postoperative period	

CHED, congenital hereditary endothelial dystrophy; DSEK, Descemet's stripping endothelial keratoplasty; PK, penetrating keratoplasty.

Expression of prostaglandin F receptor in scleral and subconjunctival tissue

Kojiro Imai, Mayumi Ueta, Kazuhiko Mori, et al.

Br J Ophthalmol 2012 96: 1148-1149 originally published online May 11, 2012
doi: 10.1136/bjophthalmol-2012-301815

Subfoveal Choroidal Thickness after Ranibizumab Therapy for Neovascular Age-related Macular Degeneration: 12-Month Results

Taizo Yamazaki, MD, Hideki Koizumi, MD, PhD, Tetsuya Yamagishi, MD, Shigeru Kinoshita, MD, PhD

Purpose: To investigate the changes in subfoveal choroidal thickness after intravitreal injections of ranibizumab (IVRs) for neovascular age-related macular degeneration (AMD).

Design: Prospective, consecutive, interventional case series.

Participants: Eighty eyes (40 affected eyes with neovascular AMD and 40 unaffected fellow eyes) of 40 patients.

Methods: Forty eyes with neovascular AMD were treated with 0.5-mg IVRs monthly for 3 months and received additional IVRs as needed over the following 9-month period. Subfoveal choroidal thickness in all 80 eyes was measured by use of enhanced depth imaging optical coherence tomography images before and after starting the IVRs.

Main Outcome Measures: Changes in subfoveal choroidal thickness after treatment by IVRs over a 12-month period.

Results: Twenty-three eyes (57.5%) were diagnosed with typical neovascular AMD, 16 eyes (40%) were diagnosed with polypoidal choroidal vasculopathy, and 1 eye (2.5%) was diagnosed with retinal angiomatous proliferation. Fifteen eyes (38%) had received some previous treatments for the neovascular lesion before undergoing the IVRs. The mean best-corrected visual acuity of the affected eyes was improved from 0.54 logarithm of the minimum angle of resolution units at baseline to 0.42 at 12 months ($P = 0.020$). The mean subfoveal choroidal thickness in the affected eyes decreased from 244±62 μm at baseline to 234±66 μm at 1 month ($P = 0.013$), 226±68 μm at 3 months ($P<0.001$), 229±67 μm at 6 months ($P = 0.002$), and 226±66 μm at 12 months ($P = 0.002$; the change ratio, 93%), whereas that in the unaffected eyes changed from 237±80 μm at baseline to 238±83 μm at 12 months ($P = 0.78$). In the affected eyes, the change ratio of subfoveal choroidal thickness at 12 months was not correlated with the number of IVRs (mean, 5.8±2.9). Subfoveal choroidal thickness demonstrated a similar trend toward decreasing during the following period independent of the subtypes of neovascular AMD or the treatment histories.

Conclusions: Subfoveal choroidal thickness decreased after IVRs in eyes with neovascular AMD. Intravitreal injections of ranibizumab may provide a pharmacologic effect not only on the neovascular lesion but also on the underlying choroid.

Financial Disclosure(s): The author(s) have no proprietary or commercial interest in any materials discussed in this article. *Ophthalmology 2012;119:1621–1627 © 2012 by the American Academy of Ophthalmology.*

Neovascular age-related macular degeneration (AMD) is a disorder associated with choroidal neovascularization (CNV) that frequently causes significant loss of vision.[1] Many kinds of therapies have been tried in an attempt to inhibit exudation induced by CNV. Ranibizumab (Lucentis; Genentech, South San Francisco, CA) is a humanized anti-vascular endothelial growth factor (VEGF) antibody fragment designed selectively to bind all forms of biologically active VEGF-A.[2,3] Intravitreal injection of ranibizumab (IVR) is currently a commonly used therapy for the treatment of neovascular AMD because IVR produces significant improvement of vision with low risks of serious systemic and ocular adverse events.[4–8] The efficacy and safety of IVR treatments have been reported for typical neovascular AMD, yet have also been reported for the other subtypes of neovascular AMD, namely, polypoidal choroidal vasculopathy (PCV)[9–13] and retinal angiomatous proliferation (RAP).[14–18]

Choroidal circulatory status seems to play an important role in the pathophysiology of neovascular AMD, for example, the findings of choroidal hypoperfusion were reportedly seen on indocyanine green angiography (ICGA) in eyes with neovascular AMD.[19–23] With regard to posttreatment hemodynamic changes in the choroid, photodynamic therapy (PDT) with verteporfin is known to induce temporary or permanent choroidal circulatory disturbances, as seen on ICGA.[24,25] However, the effect on the choroid induced by the therapeutic intervention of anti-VEGF medications has yet to be elucidated.

ISSN 0161-6420/12/$–see front matter
doi:10.1016/j.ophtha.2012.02.022

The imaging method known as enhanced depth imaging (EDI) optical coherence tomography (OCT) was recently introduced,[26] enabling clinicians to visualize the cross-sectional structure of the choroid. Several previous studies have reported successful measurements of the choroidal thickness using EDI OCT in normal eyes,[26,27] as well as in eyes with high myopia,[28] typical neovascular AMD,[29,30] PCV,[29–31] central serous chorioretinopathy,[32] Vogt–Koyanagi–Harada disease,[33] idiopathic macular hole,[34] and other disorders.[35–38]

Although IVR is widely understood to be effective for the resolution of pathologic fluid accumulation caused by CNV, little is known about the direct influences of IVR on the choroid under the CNV. In an attempt to assess the effect of IVR on the choroid in vivo, the current study investigated the changes in subfoveal choroidal thickness in eyes with neovascular AMD after IVRs over a 12-month period and compared them with those in the unaffected fellow eyes.

Patients and Methods

This prospective and interventional case series study involved 40 consecutive patients with neovascular AMD who were initially seen at the Macula Service of Kyoto Prefectural University of Medicine between March 2009 and May 2010. Each diagnosis of the subtype of neovascular AMD (typical neovascular AMD, PCV, and RAP) was based on the funduscopic and angiographic findings. Typical neovascular AMD was characterized by exudative changes due to CNV revealed by fluorescein angiography (FA) and ICGA. The diagnosis of PCV was based on ICGA findings, which demonstrated polypoidal structures at the border of the branching choroidal vascular networks.[39] In some cases, subpigment epithelial orange-red protrusions were biomicroscopically seen that corresponded to the polypoidal lesions revealed by ICGA. The diagnosis of RAP was based on the characteristic features, including intraretinal hemorrhage, intraretinal vascular anastomoses, and the OCT appearance of retinal pigment epithelial detachment with overlying cystic retinal edema.[40] Patients were excluded if their affected eyes had CNV secondary to other macular disorders, such as angioid streaks, or their fellow eyes had any macular disorder with visual loss. Patients were also excluded if each of the patients' eyes had any of the following criteria: (1) a spherical equivalent of −6 diopters (D) or less or chorioretinal atrophic changes secondary to pathologic myopia; (2) a history of intraocular surgery within 6 months; and (3) a history of pars plana vitrectomy. In addition, patients with systemic contraindication for IVRs were also excluded.

At baseline, all 40 patients underwent comprehensive ophthalmic examinations including refraction, best-corrected visual acuity (BCVA) testing with Landolt C charts, slit-lamp biomicroscopy with contact or noncontact lenses, color fundus photography, FA and ICGA using a confocal scanning laser ophthalmoscopy (Heidelberg Retina Angiograph II; Heidelberg Engineering Inc., Dossenheim, Germany), and a spectral-domain OCT (3D-OCT 1000 Mark II; Topcon Corporation, Tokyo, Japan). At each monthly visit over a 12-month period, all patients underwent BCVA testing, slit-lamp biomicroscopy, color fundus photography, and OCT imaging. The EDI-OCT images of the affected eyes with neovascular AMD were obtained at baseline, 1 month, 3 months, 6 months, and 12 months, and those of their unaffected fellow eyes were obtained at baseline and at 12 months as a control. Fluorescein angiography and ICGA were performed at 3 and 12 months and if deemed necessary.

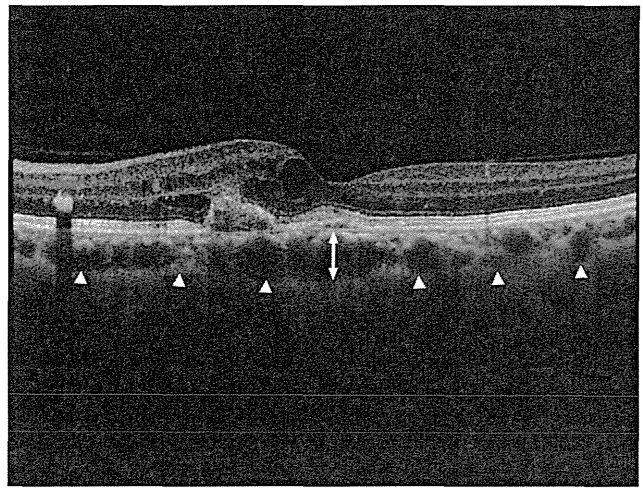

Figure 1. Enhanced depth imaging optical coherence tomography showing the retinal pigment epithelial detachment and the macular edema caused by polypoidal choroidal vasculopathy. *Double-headed arrow* indicates the subfoveal choroidal thickness. *Arrowheads* indicate the inner surface of the sclera.

The method used in the present study for obtaining the EDI OCT images was the same as reported previously.[26,29] Briefly, the choroid was imaged by positioning a spectral-domain OCT instrument close enough to the eye to obtain an inverted image. By means of the choroidal mode within the OCT that was used, the inverted images automatically appeared on the monitor to match those seen with the conventional imaging. The 6-mm horizontal and vertical scans, each comprising a maximum of 16 averaged scans, were obtained through the center of the fovea. Next, the 1 scan of 2 scans in which the inner surface of the sclera was more clearly visualized was selected. Subfoveal choroidal thickness was defined as the distance between the hyperreflective line corresponding to Bruch's membrane beneath the retinal pigment epithelium and the inner surface of the sclera by using the caliper function of the OCT device (Fig 1). The reliability of the choroidal thickness measurement method applied in the present study has been validated by the findings in our previous report.[29] All of the measurements were obtained by investigators who were masked to the patients' information.

All 40 patients were administered a 0.5-mg IVR monthly for 3 months: at baseline, at 1 month, and at 2 months. From 3 months onward, the patients received additional IVRs if any of the following criteria were observed: (1) persistent or recurrent subretinal or intraretinal fluid detected by OCT, (2) a new macular hemorrhage, (3) a new evolution of classic CNV, or (4) an expanding pigment epithelial detachment.

The primary outcomes were the changes in subfoveal choroidal thickness in the affected eyes treated with IVRs and those in the unaffected fellow eyes. The change ratio of subfoveal choroidal thickness at each time point to that at baseline was then calculated. When no change of subfoveal choroidal thickness was induced by IVRs, the change ratio was considered to be 100%.

The data obtained from all patients were analyzed with frequency and descriptive statistics. Mean values were compared by use of the Mann–Whitney *U* test or a Wilcoxon signed-rank test. The correlations between patient age or the spherical equivalent and subfoveal choroidal thickness, as well as the association between the change ratio of subfoveal choroidal thickness at 12 months and the number of IVRs administered, were assessed by use of the Spearman's rank correlation test. The differences in

outcomes among the neovascular lesions categorized by FA were compared by use of the Kruskal–Wallis test. The BCVA was converted to the logarithm of the minimum angle of resolution (logMAR) units before the calculations. Data were expressed as mean ± standard deviation, and a P value less than 0.05 was considered to be significant. All statistical analyses were performed with Statcell software version 1.0 (OMS Publishing Inc., Saitama, Japan). The study protocol followed the tenets of the Declaration of Helsinki and was approved by the institutional review board of Kyoto Prefectural University of Medicine.

Results

Eighty eyes (40 affected eyes with neovascular AMD and 40 unaffected fellow eyes) of 40 patients (16 female [40%] and 24 male [60%]; mean age: 70.7±7.7 years; range, 52–89 years) were evaluated in this study. Original refractive errors could not be identified for 6 affected eyes and 4 unaffected eyes that had undergone cataract surgery elsewhere more than 6 months before examination. Therefore, with the exception of pseudophakic eyes, the mean spherical equivalents of the remaining 34 affected eyes and 36 unaffected eyes were 0.13±1.44 D (range, −2.50 to 3.63 D) and 0.55±1.46 D (range, −2.63 to 3.50 D), respectively, with no significant difference (P = 0.16). Before the administration of the IVRs, 15 of the 40 affected eyes (38%) had received some previous treatment for neovascular lesion, including 1 to 2 sessions of PDT monotherapy (9 eyes), PDT combined with an intravitreal bevacizumab injection (IVB) (2 eyes), 1 to 2 sessions of IVB alone (3 eyes), and conventional laser photocoagulation (1 eye). At baseline, the neovascular lesions were categorized by FA as predominantly classic type (8 eyes, 20%), minimally classic type (10 eyes, 25%), and occult with no classic type (22 eyes, 55%). As to the subtypes of the neovascular lesions, 23 eyes (57.5%) were diagnosed with typical neovascular AMD, 16 eyes (40%) were diagnosed with PCV, and 1 eye (2.5%) was diagnosed with RAP. The mean number of IVRs that were administered over the 12-month period was 5.8±2.9 (range, 3–12).

The mean logMAR BCVA of the 40 affected eyes was 0.54 (median, 0; range, −0.08 to 2.00) at baseline, 0.50 (median, 0.46; range, −0.08 to 2.00) at 1 month, 0.45 (median, 0.40; range, −0.08 to 2.00) at 3 months, 0.42 (median, 0.40; range, −0.18 to 2.00) at 6 months, and 0.42 (median, 0.35; range, −0.08 to 1.40) at 12 months. Compared with that at baseline, the mean BCVA at 1 month, 3 months, 6 months, and 12 months were all significantly improved (P = 0.026, 0.006, 0.005, and 0.020, respectively). The mean logMAR BCVA of the unaffected eyes was 0.004 (median, 0.00; range, −0.18 to 0.70) at baseline.

Of the 40 affected eyes, the mean subfoveal choroidal thickness as measured by use of the obtained EDI-OCT images was significantly reduced from 244±62 μm at baseline to 234±66 μm (change ratio compared with baseline, 96%) at 1 month (P = 0.013), 226±68 μm (92%) at 3 months (P<0.001), 229±67 μm (94%) at 6 months (P = 0.002), and 226±66 μm (93%) at 12 months (P = 0.002) (Fig 2), whereas that of the 40 unaffected eyes was basically unchanged from 237±80 μm at baseline to 238±83 μm (100%) at 12 months (P = 0.78). Subfoveal choroidal thickness of the affected eyes at baseline was not correlated with patient age and spherical equivalent (rs = −0.005, P = 0.97, and rs = 0.31, P = 0.069, respectively). In addition, subfoveal choroidal thickness of the unaffected eyes at baseline was not correlated with age or spherical equivalent (rs = −0.055, P = 0.73, and rs = 0.31, P = 0.070, respectively). In the affected eyes, the change ratio of subfoveal choroidal thickness at 12 months to that at baseline was not correlated with the number of IVRs administered over the 12-month period (rs = 0.15, P = 0.42).

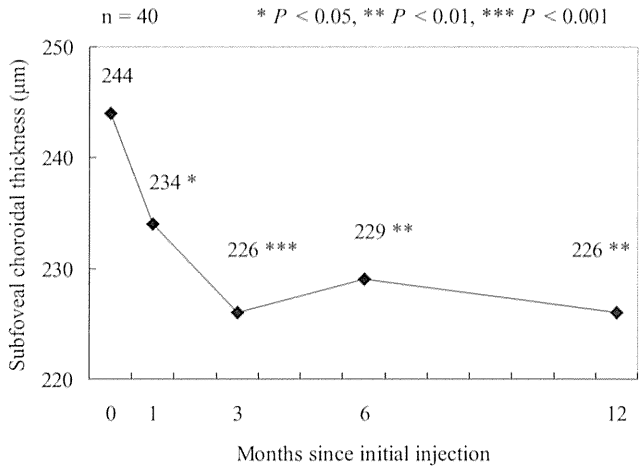

Figure 2. Changes in the mean subfoveal choroidal thickness in 40 eyes with neovascular age-related macular degeneration over the 12-month period after the initial intravitreal injections of ranibizumab. The mean subfoveal choroidal thickness significantly decreased at 1, 3, 6, and 12 months compared with baseline (0).

In regard to the subtypes of neovascular AMD, the mean subfoveal choroidal thickness at baseline was 235±65 μm in 23 eyes with typical neovascular AMD and 261±54 μm in 16 eyes with PCV. However, the difference between the 2 subtypes was not significant (P = 0.21). After starting IVRs, subfoveal choroidal thickness in eyes with typical neovascular AMD and in eyes with PCV demonstrated a similar trend toward decreasing over the following 12-month period. The mean subfoveal choroidal thickness in eyes with typical neovascular AMD was 229±72 μm (change ratio compared with baseline, 97%) at 1 month, 215±73 μm (90%) at 3 months, 220±68 μm (93%) at 6 months, and 218±65 μm (93%) at 12 months. Compared with baseline, the mean subfoveal choroidal thickness at 3 months, 6 months, and 12 months was significantly reduced (P = 0.005, 0.007, and 0.007, respectively). The mean subfoveal choroidal thickness in 16 eyes with PCV was 245±57 μm (94%) at 1 month, 246±56 μm (95%) at 3 months, 247±64 μm (95%) at 6 months, and 243±68 μm (93%) at 12 months. Compared with baseline, the mean subfoveal choroidal thickness significantly decreased at 1 month (P = 0.028) and 3 months (P = 0.047). In 1 eye with RAP that had previously received PDT combined with an IVB, subfoveal choroidal thickness was 177 μm at baseline and decreased to 163 μm (92%) at 1 month, 159 μm (90%) at 3 months, 157 μm (89%) at 6 months, and 159 μm (90%) at 12 months. These results are summarized in Figure 3.

The mean subfoveal choroidal thickness at baseline was 245±66 μm in 25 eyes without previous treatments and 242±56 μm in 15 eyes with previous treatments, with no significant difference found between the 2 groups (P = 0.75). The mean subfoveal choroidal thickness of those 2 groups demonstrated a similar trend toward decreasing over the following 12-month period. The mean subfoveal choroidal thickness in eyes without previous treatments was 233±74 μm (change ratio compared with baseline, 94%) at 1 month, 227±76 μm (92%) at 3 months, 228±73 μm (93%) at 6 months, and 229±71 μm (93%) at 12 months. Compared with baseline, the mean subfoveal choroidal thickness at all 4 time points was significantly reduced (P = 0.038, 0.013, 0.008, and 0.024, respectively). The mean subfoveal choroidal thickness in eyes with previous treatments was 235±52 μm (98%) at 1 month, 223±54 μm (93%) at 3 months, 230±59 μm (95%) at 6 months, and 222±59 μm (92%) at 12 months. Compared with

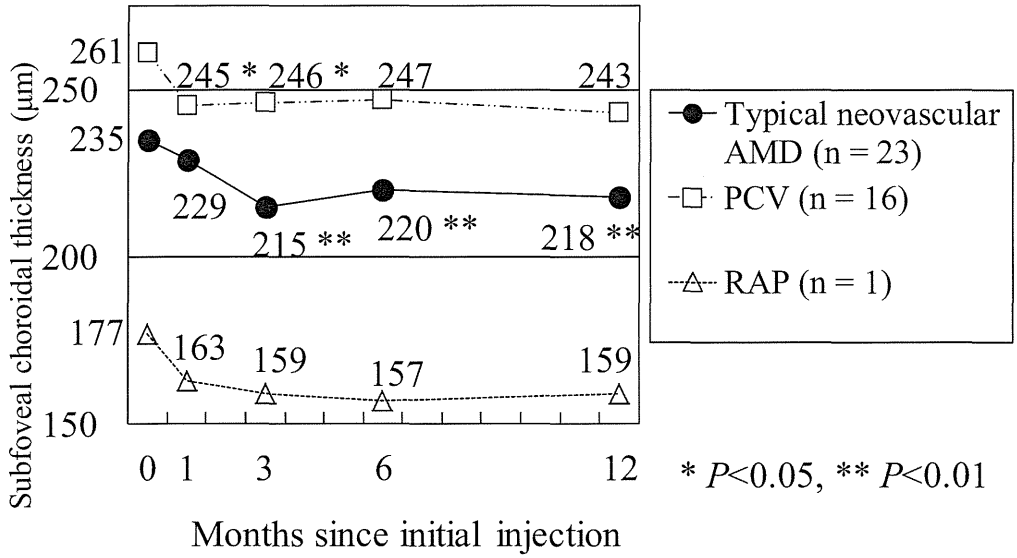

Figure 3. Changes in the mean subfoveal choroidal thickness in eyes with typical neovascular age-related macular degeneration (AMD), polypoidal choroidal vasculopathy (PCV), and retinal angiomatous proliferation (RAP) over the 12-month period after the initial intravitreal injections of ranibizumab. The subfoveal choroidal thickness in the 3 subtypes demonstrated a similar trend toward decreasing during the 12-month period. The mean subfoveal choroidal thickness in eyes with typical neovascular AMD at 3, 6, and 12 months significantly decreased compared with that at baseline (0). The mean subfoveal choroidal thickness in eyes with PCV was significantly reduced at 1 and 3 months compared with that at baseline.

baseline, the mean subfoveal choroidal thickness at 3 months and 12 months was significantly reduced (P = 0.013 and 0.023, respectively). These results are summarized in Figure 4. None of the 40 patients developed severe systemic or ocular complications related to IVRs.

Discussion

To the best of our knowledge, the changes in choroidal thickness during IVR therapy have not been reported. This is the first study to show the decreased subfoveal choroidal thickness in eyes with neovascular AMD treated with IVRs.

The mean subfoveal choroidal thickness in all 40 eyes treated with IVRs decreased from 244 μm at baseline to 226 μm at 3 months, which remained until 12 months. The rate of decrease in subfoveal choroidal thickness was 18 μm/year. The rate was greater than that reported in normal eyes (1.56 μm/year),[27] and results of the present study indicate the possibility that IVRs influenced the choroidal structure under the neovascular membrane in neovascular AMD. The

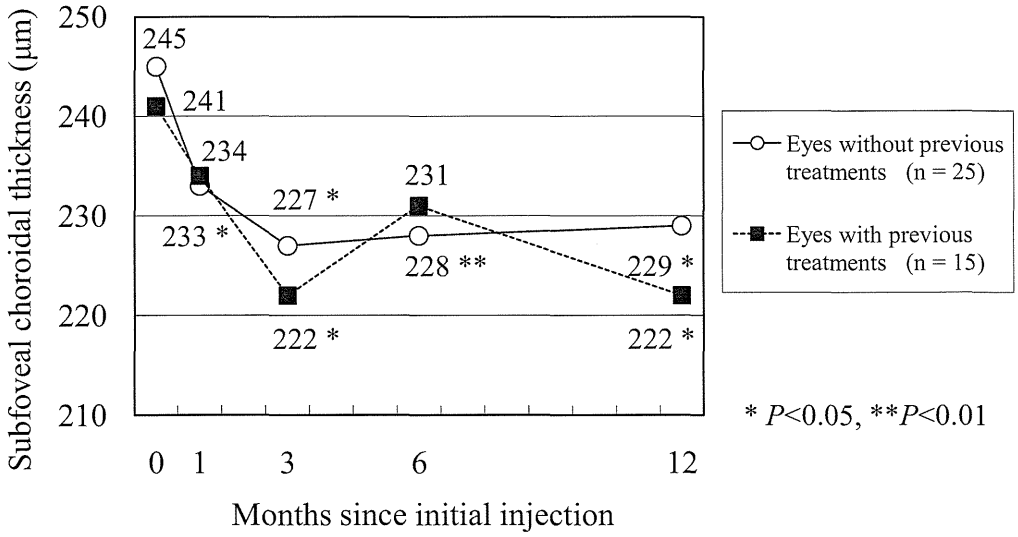

Figure 4. Change in the subfoveal choroidal thickness in eyes with or without previous treatments for neovascular age-related macular degeneration over the 12-month period after the initial intravitreal injections of ranibizumab. The mean subfoveal choroidal thickness in the 2 groups demonstrated a similar trend toward decreasing during the 12-month period. The mean subfoveal choroidal thickness in eyes without previous treatments significantly decreased at all 4 time points compared with that at baseline. The mean subfoveal choroidal thickness in eyes with previous treatments significantly decreased at 3 and 12 months compared with that at baseline (0).

mean subfoveal choroidal thickness after IVRs significantly decreased at 1 month and reached the maximum amount of decrease at 3 months. This trend might implicate that 1 injected dose of ranibizumab (0.5 mg) was sufficient to cause the significant decrease in choroidal thickness and that the additional 2 monthly administrations of IVRs had an additive effect. However, the correlation between the number of IVRs administered over the 12-month period and the change ratio of subfoveal choroidal thickness was not significant. Therefore, the findings of this study indicate that 3 monthly injections of IVRs were sufficient for the choroidal thickness to reach the maximum amount of decrease at 3 months that plateaued and remained unchanged independently of the number of additional IVRs.

Maruko and associates[31] recently reported that in both the PDT monotherapy and the combined therapy of PDT and IVR for PCV, subfoveal choroidal thickness transiently increased 2 days after PDT, followed by its significant decrease through 6 months. However, the authors did not comment on the additional effect of IVRs on the changes in subfoveal choroidal thickness. The analyses of data presented in their report revealed that the mean subfoveal choroidal thickness in 11 patients treated with combined therapy had slightly decreased from 264 μm to 262 μm in 1 or 2 days after IVR that preceded PDT.[31] In the current study, subfoveal choroidal thickness in eyes with PCV significantly decreased from 261 μm at baseline to 245 μm at 1 month and remained unchanged over 12 months. Our data may suggest that not only PDT but also IVRs may have an effect to decrease the choroidal thickness in eyes with PCV, although the rate of the reduction induced by IVR alone seemed to be less than that by PDT.[31] The difference in the degree of choroidal thickness decrease between PDT and IVR might be attributable to the different action mechanisms between these 2 treatments because PDT reportedly induces photothrombotic choroidal vascular occlusion.[24,25]

Recent studies have reported that eyes with PCV have a thicker choroid under the fovea than those with typical neovascular AMD.[29,30] In the present study, subfoveal choroidal thickness in eyes with PCV at baseline was not significantly thicker than that in eyes with typical neovascular AMD, possibly because of the influence on the choroid by the various previous treatments for the 7 eyes with typical AMD and the 7 eyes with PCV. Subfoveal choroidal thickness in eyes with the 3 subtypes of neovascular AMD, namely, typical neovascular AMD, PCV, and RAP, showed a similar trend toward decreasing during the following 12-month period, which may implicate that IVR has a constant effect on the choroid, regardless of the subtype of neovascular AMD and the treatment histories.

Vascular endothelial growth factor-A provides various pharmacologic actions on the choroid, such as increases in microvascular permeability, angiogenesis, and survival for the vascular endothelial cells.[2,3] Vascular endothelial growth factor-A is also considered to play a key role in the pathogenesis of neovascular AMD. In fact, a significantly increased expression of VEGF was measured in the aqueous humor of eyes with neovascular AMD, and a significant decrease of VEGF was observed after IVRs.[41] In rabbits, ranibizumab rapidly penetrates after intravitreal injection through all retinal layers to reach the choroid[42] and may have the potential to inhibit the activity of all VEGF-A isoforms.[2,3] One reason for the decreased choroidal thickness in the current study might be related to the reduction of choroidal vascular permeability, as seen in eyes with central serous chorioretinopathy treated with IVBs.[43] However, we were not able to draw a conclusion on the other reasons, such as morphologic changes in the choroidal vasculature itself, because to date it remains unclear whether IVR has a potential to provide a vasoconstriction effect on the choroidal vasculature similar to that reportedly seen in the retinal vasculature.[44,45] Further investigation on the cross-sectional choroidal structure using higher-resolution instruments may elucidate the reason why the choroidal thickness in neovascular AMD decreases after IVRs.

Study Limitations

The current study has several limitations, such as the limited number of cases that were included. The subfoveal choroidal thickness was investigated at the fixed time points; however, the relationship between subfoveal choroidal thickness and the disease activities still remains unknown. Although the measurements were performed by investigators masked to the patients' information, those measurements were performed manually. Future studies with automated software will be required for a more objective evaluation.

In conclusion, subfoveal choroidal thickness appeared to decrease after IVRs in eyes with neovascular AMD. Intravitreal injections of ranibizumab may provide a pharmacologic effect not only on the CNV but also on the choroid under the neovascular lesion. It may be intriguing to investigate which other anti-VEGF medications have a similar effect on the choroidal thickness to further understand their pharmacokinetics and the pathophysiology of neovascular AMD.

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Footnotes and Financial Disclosures

Originally received: October 10, 2011.
Final revision: December 19, 2011.
Accepted: February 15, 2012.
Available online: May 1, 2012. Manuscript no. 2011-1485.

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Presented at: The 2011 Annual Meeting of the American Academy of Ophthalmology (PO510), October 24, 2011, Orlando, Florida.

Financial Disclosure(s):
The author(s) have no proprietary or commercial interest in any materials discussed in this article.

This study was supported in part by grant no. 21890226 from the Ministry of Education, Culture, Sports, Science and Technology-Japan (Dr. Koizumi).

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Adv Ther (2012) 29(4):339-349.
DOI 10.1007/s12325-012-0016-x

ORIGINAL RESEARCH

Comparison of Corneal and Aqueous Humor Penetration of Moxifloxacin, Gatifloxacin and Levofloxacin During Keratoplasty

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Received: December 4, 2011 / Published online: April 11, 2012

ABSTRACT

Introduction: Achieving high antibiotic concentrations is important for preventing and treating postoperative infections. However, no study has simultaneously compared the achieved concentrations of moxifloxacin, gatifloxacin, and levofloxacin in the human cornea and aqueous humor. The authors therefore performed a randomized study to determine the concentrations of 0.5% moxifloxacin, 0.3% gatifloxacin, and 0.5% levofloxacin in the corneal tissue and aqueous humor after topical instillation in patients undergoing penetrating keratoplasty.

Methods: Patients who required penetrating keratoplasty were eligible for this study. The topical preparations of 0.5% moxifloxacin, 0.3% gatifloxacin, and 0.5% levofloxacin used in the study were preservative free (Japanese formulations). Patients were randomly assigned to one of three sequential drug groups, in which each drug was administered three times before surgery. In each administration cycle,

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the patients received two drops of each drug at 2-minute intervals. Samples of corneal tissue and aqueous humor were collected during surgery. The concentrations of each drug in the samples were determined by high-performance liquid chromatography.

Results: A total of 63 patients across eight centers in Japan were enrolled in the study. Overall, 61 corneal and 58 aqueous humor samples were evaluated. The concentration (mean ± standard deviation) of moxifloxacin in corneal tissues was 12.66 ± 8.93 μg/g, which was significantly higher than that of gatifloxacin (4.71 ± 3.39 μg/g; $P < 0.0001$) and levofloxacin (5.95 ± 4.02 μg/g; $P < 0.0001$). The mean concentration of moxifloxacin in aqueous humor samples was 1.40 ± 1.17 μg/mL, which was significantly higher than that of gatifloxacin (0.65 ± 0.80 μg/mL; $P = 0.0001$) and levofloxacin (0.89 ± 0.86 μg/mL; $P < 0.05$). The sequence of drug administration did not significantly affect the results.

Conclusion: These results show that 0.5% moxifloxacin achieved superior ocular concentration than both 0.3% gatifloxacin and 0.5% levofloxacin.

Keywords: Aqueous humor; Concentration; Cornea; Fluoroquinolone; Gatifloxacin; Levofloxacin; Moxifloxacin

INTRODUCTION

Compared with previous generations, the fourth-generation fluoroquinolones possess greater antibiotic activity and efficacy against gram-positive and gram-negative ocular pathogens. The fourth-generation fluoroquinolones are also particularly effective against proinflammatory pathogens responsible for many ocular infections [1–4]. The fourth-generation fluoroquinolones moxifloxacin and gatifloxacin and the third-generation fluoroquinolone levofloxacin are widely used in Japan for perioperative prophylaxis and to treat ocular infections. While these drugs have similar antibiotic activities, their pharmacokinetic properties and minimum inhibitory concentrations (MICs) against individual bacteria are different. To evaluate the efficacy of an antibiotic, its potency and achieved intraocular concentration need to be considered [5]. Previous studies have shown that the concentrations of moxifloxacin are higher than those of gatifloxacin and levofloxacin in albino rabbit eyes [6, 7] and those of gatifloxacin in human eyes [8–10]. Although high antibiotic concentrations as well as antibiotic activities are important in the treatment of postoperative infections, no study has simultaneously compared the concentrations of moxifloxacin, gatifloxacin, and levofloxacin in the human cornea and aqueous humor. In addition, the topical preparations commonly used in Japan (0.3% gatifloxacin and 0.5% levofloxacin) differ from those commonly used in other countries because they lack benzalkonium chloride (BAK). In fact, Owen et al. reported that the tissue levels of levofloxacin and gatifloxacin in rabbits differed between the United States and Japanese formulations [11]. For this reason, the present authors conducted a prospective multicenter randomized trial to determine the concentration of these drugs (Japanese formulations) in the cornea and aqueous humor of patients undergoing penetrating keratoplasty (PKP).

METHODS

Study Design

A randomized multicenter collaborative study was conducted to determine the concentrations of 0.5% moxifloxacin, 0.3% gatifloxacin, and

0.5% levofloxacin ophthalmic solutions in the corneal tissue and aqueous humor following ocular instillation in patients undergoing PKP. The study protocol was based on the previously published protocols for clinical trials conducted by Yamada et al. [12, 13] and was approved by the ethics committee of each participating center. All participants gave written informed consent.

The authors enrolled patients scheduled to undergo PKP. Exclusion criteria included the presence of a corneal ulcer, persistent corneal epithelium defects, topical and/or systemic administration of fluoroquinolones or any medication that might affect the accuracy of the study assessment within a week prior to the surgery, known allergy or sensitivity to any component of the study medications, inability to provide written informed consent, and any other criteria that the physician deemed appropriate for exclusion. We complied with all applicable institutional and governmental regulations concerning the ethical use of human volunteers.

Topical preparations of 0.5% moxifloxacin (Vegamox®; Alcon Co., Ltd., Tokyo, Japan), 0.3% gatifloxacin (Gatiflo®; Senju Pharmaceutical Co., Ltd., Osaka, Japan), and 0.5% levofloxacin (Cravit®; Santen Pharmaceutical Co., Ltd., Osaka, Japan) were obtained from their manufacturers. None of the preparations contained BAK.

Patients were randomly assigned to one of three groups, in which the three drugs were administered sequentially in a crossover setting, as follows: group 1, moxifloxacin, gatifloxacin, and levofloxacin (M/G/L); group 2, gatifloxacin, levofloxacin, and moxifloxacin (G/L/M); and group 3, levofloxacin, moxifloxacin, and gatifloxacin (L/M/G). Each drug was administered three times every 15 minutes within the 30-minute period running from 90 to 60 minutes before surgery (Fig. 1). In each

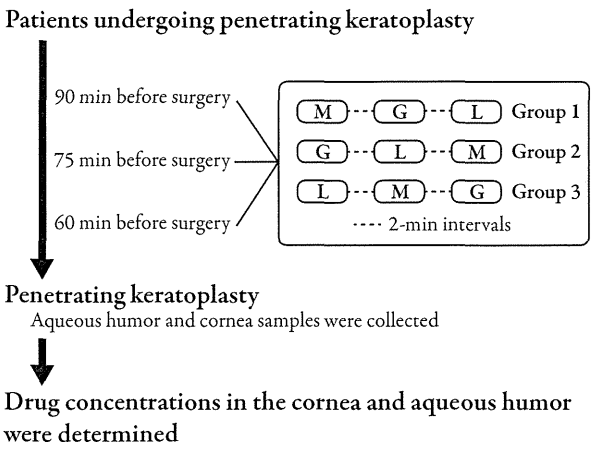

Fig. 1 Study protocol. *G* gatifloxacin, *L* levofloxacin, *M* moxifloxacin, *min* minutes

administration cycle, patients received two drops of each drug at 2-minute intervals. The drugs were administered by the physicians or trained medical staff in strict compliance with the study protocol. The subjects were instructed to keep their eyes closed after each drug administration.

Patients were scheduled to undergo surgery 60 minutes after the last dose. At the start of surgery, 0.1 mL of aqueous humor was aspirated from the anterior chamber using a syringe with a 27-gauge cannula. The host corneal button was excised, divided into halves using a razor blade, and then blotted dry using a cellulose sponge. The tissues were stored at –20°C until analysis.

Analysis

All corneal tissue samples were weighed and cut into small pieces and then homogenized in 0.8 mL of 0.1 mol/L phosphate buffer (pH 7.0). For analysis, the drugs were extracted with 6 mL of chloroform, followed by brief centrifugation. The chloroform layer was concentrated by evaporation under N_2 gas (at 40°C) and reconstituted with 0.5 mL of 0.05 mol/L phosphate buffer (pH 3.0)/acetonitrile (75:25, v/v) to obtain the final extract.

The concentrations of each drug in the corneal tissue and aqueous humor samples were determined by high-performance liquid chromatography with a pump (L-7100), fluorescence detector (L-7485), and autosampler (L-7200) from Hitachi, Ltd. (Tokyo, Japan). Data integration and manipulation were performed using EZ-Chrom Elite software (Scientific Software, Inc., San Ramon, CA, USA). Chromatographic separation was performed on a TSKgel ODS-80Tm column (4.6 mm × 250 mm, 5 μm; Tosoh, Inc., Tokyo, Japan). The mobile phase consisted of 0.05 M phosphate buffer (pH 3.0)/acetonitrile (75:25, v/v). The flow rate was 0.8 mL/min. The column was maintained at 40°C. The wavelengths of the fluorescence detector were set to 290 nm (excitation) and 470 nm (emission). The drug concentrations were determined from standard curves generated using known concentrations (0.001–0.25 μg) of the respective drug per weight of tissue or volume of aqueous humor used for this assay and the peak height corresponding to each concentration on high-performance liquid chromatography. Concentrations are expressed as micrograms of drug per gram of corneal tissue or per milliliter of aqueous humor.

The primary outcome was the concentration of each drug in the cornea and aqueous humor. Secondary outcomes were the factors that affected the concentrations of each drug. The data analysis committee, which was blinded to the treatment allocations, reviewed all outcomes and used prerandomization patient data collected before the end of the study to determine the analysis settings and thereby prevent bias. The results are presented as means ± standard deviation (SD). Analysis of variance (ANOVA) and Tukey's multiple comparison test were used to compare groups. Statistically significant differences were accepted at values of $P < 0.05$.

RESULTS

Between April 1 and December 31, 2007, 63 patients (age range, 27–82 years; mean ± SD age, 63.0 ± 15.3 years) were enrolled across eight centers in Japan. Patients were randomly assigned to three groups: group 1 (M/G/L; n = 20; 13 men and 7 women; mean age, 64.5 ± 15.5 years), group 2 (G/L/M; n = 21; 9 men and 12 women; mean age, 61.9 ± 14.7 years), and group 3 (L/M/G; n = 22; 12 men and 10 women; mean age, 62.8 ± 14.7 years). Primary indications for PKP included bullous keratopathy (n = 23), regraft (n = 17), corneal leukoma (n = 15), keratoconus (n = 6), and corneal dystrophy (n = 2). The authors did not observe any significant differences in sex, age, history of cataract extraction, primary indications for PKP, comorbidity of corneal edema, or other complications among the three groups (Table 1).

Corneal tissue and aqueous humor samples were collected from 63 and 60 patients, respectively. Although patients were scheduled to undergo surgery 60 minutes after the last dose, the actual duration between the last dose and the collection of aqueous humor samples ranged from 17 to 86 minutes. Therefore, the data analysis committee decided to limit data analysis to samples collected within 60 ± 20 minutes from the last administration. As a result, samples from two patients were excluded from the analysis of the ocular concentrations. Thus, a total of 61 corneal tissue and 58 aqueous humor samples were evaluated. These samples were collected at 54.2 ± 10.3 minutes (range, 41–77 minutes) after the last dose.

The order of drug administration did not significantly affect the achieved concentrations in any group (Table 2). The presence of superficial keratopathy or apparent corneal edema did not significantly affect the concentrations in

Table 1 Baseline characteristics of 63 patients undergoing penetrating keratoplasty

	Group 1 (M/G/L) $n = 20$	Group 2 (G/L/M) $n = 21$	Group 3 (L/M/G) $n = 22$	Total	*P* values
Sex					
Male	13	9	12	34	0.3631[a]
Female	7	12	10	29	
Age (years), mean ± SD	64.5 ± 15.5	61.9 ± 14.7	62.8 ± 14.7	63.0 ± 15.3	0.8582[b]
Primary indications for PKP					
Keratoconus	3	2	1	6	
Corneal leukoma	4	7	4	15	
Corneal dystrophy	0	1	1	2	0.5278[a]
Regraft	8	3	6	17	
Bullous keratopathy	5	8	10	23	
Prominent corneal edema					
Present	11	11	17	39	0.1823[a]
Absent	9	10	5	24	
Combined cataract extraction					
Performed	9	6	10	25	0.7449[a]
Not performed	11	15	12	38	
Superficial punctate keratopathy					
Present	4	7	6	17	0.6295[a]
Absent	16	14	16	46	

[a] χ^2 test
[b] ANOVA (analysis of variance)
G gatifloxacin, *L* levofloxacin, *M* moxifloxacin, *PKP* penetrating keratoplasty

Table 2 Effects of drug order on achieved concentrations of fluoroquinolones in 63 patients undergoing penetrating keratoplasty

	Group 1 (M/G/L)	Group 2 (G/L/M)	Group 3 (L/M/G)	*P* value[a]
Cornea (μg/g) ($n = 61$)				
Number of patients	19	21	21	
Moxifloxacin	12.68 ± 5.88	13.93 ± 13.00	11.37 ± 6.00	0.6592
Gatifloxacin	4.49 ± 2.22	4.12 ± 4.22	5.49 ± 3.34	0.4057
Levofloxacin	7.25 ± 3.56	5.06 ± 4.77	5.67 ± 3.44	0.2118
Aqueous humor (μg/mL) ($n = 58$)				
Number of patients	19	19	20	
Moxifloxacin	1.30 ± 1.12	1.28 ± 0.79	1.61 ± 1.51	0.6214
Gatifloxacin	0.48 ± 0.43	0.49 ± 0.43	0.98 ± 1.12	0.0727
Levofloxacin	0.93 ± 0.87	0.63 ± 0.47	1.12 ± 1.09	0.2074

[a] ANOVA (analysis of variance)
G gatifloxacin, *L* levofloxacin, *M* moxifloxacin
Values are means ± SD

either aqueous humor or corneal tissue (data not shown). The concentrations of the three fluoroquinolones in the same samples correlated well with each other. In the corneal tissue the correlation coefficient for moxifloxacin and gatifloxacin was 0.78, and that for moxifloxacin and levofloxacin was 0.77. In the aqueous humor the correlation coefficient for moxifloxacin and gatifloxacin was 0.86, and that for moxifloxacin and levofloxacin was 0.89.

Moxifloxacin concentrations in both aqueous humor and corneal tissue were significantly higher than those of gatifloxacin and levofloxacin (Table 3 and Fig. 2).

Table 3 Corneal and aqueous humor concentrations of fluoroquinolones in 63 patients undergoing penetrating keratoplasty

	Mean ± SD (μg/g)	Median (range) (μg/g)	*P* values[a]		
Cornea ($n = 61$)					
Moxifloxacin	12.66 ± 8.93	10.53 (0.69–59.91)	M versus L: < 0.0001	L versus G: 0.4855	G versus M: <0.0001
Levofloxacin	5.95 ± 4.02	5.25 (0.56–23.48)			
Gatifloxacin	4.71 ± 3.39	4.44 (0.30–20.84)			
Aqueous humor ($n = 58$)					
Moxifloxacin	1.40 ± 1.17	1.08 (0.16–7.12)	M versus L: 0.0138	L versus G: 0.3738	G versus M: 0.0001
Levofloxacin	0.89 ± 0.86	0.59 (0.09–4.51)			
Gatifloxacin	0.65 ± 0.80	0.41 (0.06–4.81)			

[a] Tukey's multiple comparison test
G gatifloxacin, *L* levofloxacin, *M* moxifloxacin

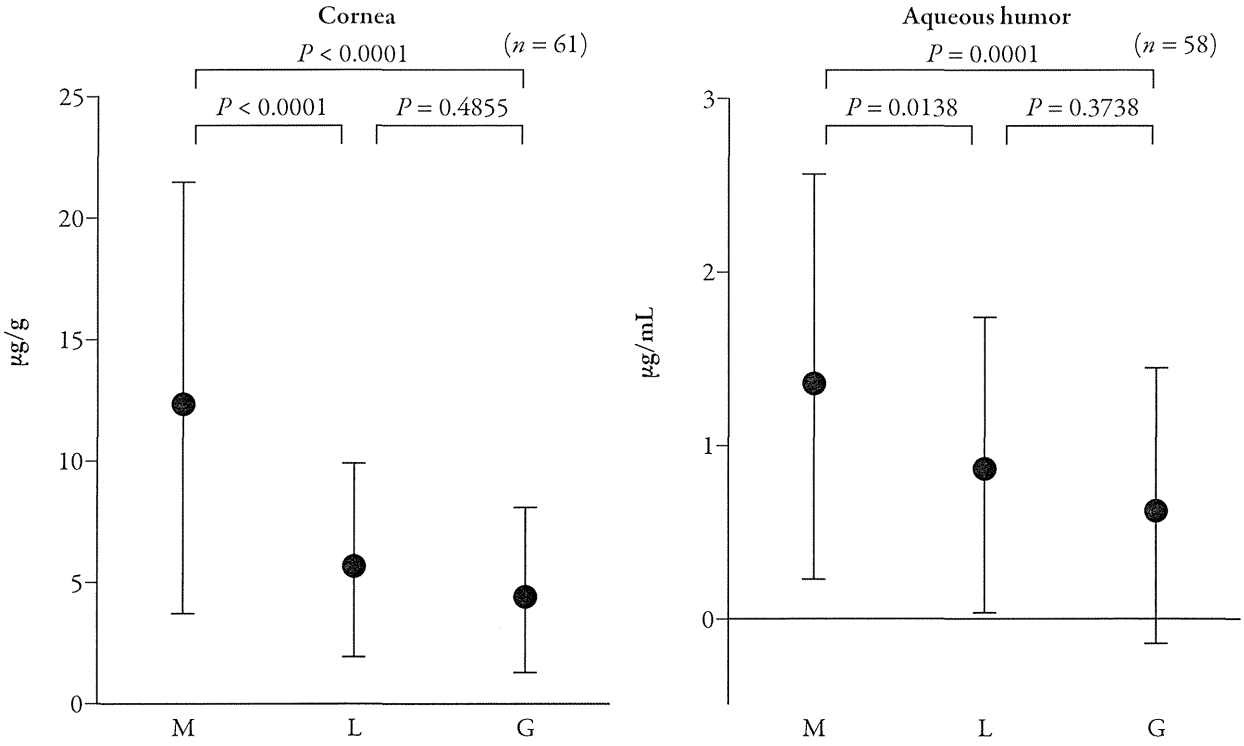

Fig. 2 Corneal and aqueous humor concentrations (mean ± SD) of the three fluoroquinolones studied. Statistical comparisons were made using Tukey's multiple comparison test. *G* gatifloxacin, *L* levofloxacin, *M* moxifloxacin

The mean concentration of moxifloxacin in corneal tissue was 12.66 ± 8.93 μg/g, which was significantly higher than that of gatifloxacin (4.71 ± 3.39 μg/g, $P < 0.0001$) or levofloxacin (5.95 ± 4.02 μg/g, $P < 0.0001$). The mean concentration of moxifloxacin in aqueous humor was 1.40 ± 1.17 μg/mL, which was significantly higher than that of gatifloxacin (0.65 ± 0.80 μg/mL, $P = 0.0001$) or levofloxacin (0.89 ± 0.86 μg/mL, $P = 0.0138$). There were no statistically significant differences between the concentrations of levofloxacin and gatifloxacin in the corneal tissue or aqueous humor.

DISCUSSION

Fluoroquinolones generally show excellent bactericidal activities against several common gram-positive and gram-negative ocular pathogens, and show high potencies [2–4, 14]. As a result, fluoroquinolones are widely used to treat ocular infections and prevent perioperative infections, including endophthalmitis. The clinical usefulness of fluoroquinolones depends on several factors, including their in-vitro bactericidal activities, their ability to penetrate the site of infection, and their relative toxicities. In particular, fluoroquinolones must penetrate the affected tissue to an appropriate level because the clinical response to these drugs is concentration-dependent. This is why the authors determined the aqueous humor and corneal tissue concentrations of two fourth-generation fluoroquinolones (0.5% moxifloxacin and 0.3% gatifloxacin) and one third-generation fluoroquinolone (0.5% levofloxacin) administered to patients 60 minutes before PKP.

The authors found marked differences in the ocular concentrations of each drug. These results are comparable with those of previous reports, which showed that the concentration of moxifloxacin in the eyes of albino rabbits was higher than that of gatifloxacin or levofloxacin [6, 7] and was higher than that of gatifloxacin in human eyes (Table 4) [9–13, 15]. All commercial formulations of fluoroquinolones currently used in Japan are free from preservatives. To the authors' knowledge, this is the first study to determine the concentrations of three BAK-free antibiotic drugs in the human eye. The higher ocular concentration of moxifloxacin relative to gatifloxacin and levofloxacin is not therefore due to the effect of preservatives, but to inherent differences in molecular structure, and to the higher fluoroquinolone concentration in topical preparations (0.3% gatifloxacin vs. 0.5% moxifloxacin and 0.5% levofloxacin). The higher lipophilicity and aqueous solubility of moxifloxacin might be responsible for the higher ocular concentration of this substance [16].

Some caution, however, should be exercised when interpreting these data. The topical preparations (0.3% gatifloxacin and 0.5% levofloxacin) used in the present study are different from those used in most other countries in that they do not contain preservatives. The addition of preservatives to an ophthalmic solution causes a number of effects. Most eyedrops contain preservatives that inhibit bacterial growth and keep the eye safe. However, the use of multiple ophthalmic drugs containing preservatives and/or the long-term use of ophthalmic solutions containing preservatives may cause damage to the ocular surface [17]. This is why there is a tendency to avoid the use of preservatives in ophthalmic preparations, especially in Japan. On the other hand, preservatives may have a beneficial effect in terms of drug penetration [11]. For example, preservatives such as BAK can actually enhance drug penetration into the cornea by disrupting the barrier function of the corneal epithelium. Owen et al. reported that the tissue levels of

Table 4 Aqueous humor and corneal drug concentrations of fluoroquinolones: summary of literature results

Study	Type of surgery (no. of patients)	Method	Sample	Moxifloxacin[a]	Gatifloxacin[a]	Levofloxacin[a]	Norfloxacin[a]	Lomefloxacin[a]
Monotherapy								
Kim et al. [8]	Cataract (50)	1 drop Q10 min preop. (4 times)	Aqueous humor	1.80 ± 1.21	0.48 ± 0.34	–	–	–
				$P = 0.0003$		–	–	–
McCulley et al. [9]	Cataract (46)	QID × 1 day + 1 drop 1 h preop. (5 times)	Aqueous humor	1.86 ± 1.06	0.94 ± 0.72	–	–	–
				$P < 0.001$		–	–	–
Holland et al. [10]	Keratoplasty (48)	1 drop Q5 min at 0.25, 0.5, 1, or 2 h preop. (2 times)	Aqueous humor	0.321 ± 0.541 to 0.716 ± 0.388	0.029 ± 0.042 to 0.327 ± 0.245	–	–	–
			Corneal epithelium	81.2 ± 87.8[b]	12.3 ± 13.5[b]	–	–	–
			Corneal stroma	48.5 ± 33.5[b]	15.7 ± 15.8[b]	–	–	–
			Corneal endothelium	76.1 ± 76.8[b]	7.3 ± 7.0[b]	–	–	–
Katz et al. [15]	Cataract (60)	1 drop Q15 min preop. (4 times)	Aqueous humor	1.50 ± 0.75	–	–	–	–
		QID × 1 day + 4 drops preop. (8 times)	Aqueous humor	1.74 ± 0.66	–	–	–	–
Combination therapy								
Yamada et al. [12]	Cataract (59)	1 drop Q15 min/ 90 min before preop. (3 times)	Aqueous humor	–	–	0.60 ± 0.28	0.01 ± 0.02	0.23 ± 0.11
Yamada et al. [13]	Keratoplasty (14)	1 drop Q15 min/ 90 min before preop. (3 times)	Cornea	–	–	4.6 ± 3.5[b]	1.3 ± 1.2[b]	2.7 ± 1.8[b]
Present study	Keratoplasty (63)	2 drops Q15 min preop. (6 times)	Cornea	12.70 ± 8.93[b]	4.71 ± 3.89[b]	5.95 ± 4.02[b]	–	–
			Aqueous humor	1.40 ± 1.17	0.65 ± 0.80	0.89 ± 0.86	–	–

[a] Concentrations are given as means ± SD (μg/mL or [b]μg/g)

Q5 every 5 min, *Q10* every 10 min, *Q15* every 15 min, *QID* 4 times daily, *SD* standard deviation, *h* hour, *min* minutes, *preop.* preoperatively

levofloxacin and gatifloxacin in a rabbit model differed between the United States and Japanese formulations [11].

Another issue is that BAK has intrinsic antimicrobial effects. Hyon et al. reported that the addition of BAK to fluoroquinolone ophthalmic solutions quickened bacterial death [18]. Eradicating bacteria from the ocular surface is a key determinant of efficient prophylaxis of postoperative ocular infections. Thus, the addition of BAK to an ocular antibiotic formulation may help to eliminate bacteria from the ocular surface and enhance drug penetration into ocular tissues. However, it is impossible to evaluate the clinical significance of BAK on the basis of the results from this study because none of the formulations used here contained BAK.

In this study, the fluoroquinolone concentrations in the cornea and aqueous humor showed substantial interpatient variability even though each patient received the same amount of each drug. Marked interpatient variability was also observed in earlier studies assessing fluoroquinolone penetration into the cornea and aqueous humor [8–10, 12, 13, 16, 18]. Multiple factors, including tear turnover rate, blinking frequency and completeness, timing of sampling, and epithelial continuity, are thought to contribute to this large interpatient variability. In the present study, the condition of the corneal epithelium might be influential, because all of the subjects were undergoing PKP [19–21]. However, the presence of superficial keratopathy or apparent corneal edema did not significantly affect the concentrations in both aqueous humor and corneal tissue in the present study. Another important factor appears to be the timing of sampling. In our study, the actual time from the last dose to sample collection ranged from 17 to 86 minutes, even though sample collection was scheduled to be performed 60 minutes after the last dose. Although the pharmacokinetic analyses performed by Fukuda and Sasaki have shown this to be an effective approach in rabbits, there is an inevitable limitation in human pharmacokinetic data because samples are typically obtained after dosing the eye before surgery [6, 11].

To minimize interpatient variability, we used the analytical method originally reported by Diamond et al. [21]. Any factors that promote or inhibit the penetration of one drug would be expected to have an essentially identical effect on all of the drugs, because all three fluoroquinolones were administered to each eye simultaneously. All three fluoroquinolones were also assayed simultaneously in each corneal and aqueous humor sample to increase the effective sample size. However, the administration of multiple eyedrops at the same time has potential drawbacks [22]. For this reason, we administered the drugs at 2-minute intervals to minimize any potential washout effects. However, we have shown that the administration of multiple eyedrops, even with a 2-minute interval [12], may result in reduced drug penetration. Although the superior penetration of moxifloxacin into ocular tissues observed here is a valid finding, the data cannot be directly compared with the results of other studies in which only a single drug was administered.

A number of fluoroquinolone-resistant bacteria have been reported in recent years [23–27]. The use of moxifloxacin is impractical for the treatment of ocular infections caused by fluoroquinolone-resistant bacteria. However, for prophylactic use, antibiotic drugs that can prevent the emergence of antibiotic-resistant bacteria are highly desired. The emergence of antibiotic-resistant bacteria is most likely to occur if the achieved concentration ranges from the MIC to the mutant prevention concentration (MPC) [28, 29]. Therefore, for sustained efficacy and to avoid the emergence of

antibiotic-resistant bacteria, both the MIC and the MPC must be exceeded. A previous intent-to-treat study, in which high aqueous humor concentrations of moxifloxacin were achieved, suggested that moxifloxacin might penetrate the aqueous humor at concentrations exceeding the MIC and MPC of most pathogenic bacteria [30]. In addition, advantages of moxifloxacin over previously available fluoroquinolones have been shown in vitro, including greater antibiotic activity [1, 2]. Thus, moxifloxacin possesses excellent sensitivity and antibiotic activity against most pathogenic bacteria, supporting its efficacy in preventing perioperative endophthalmitis. These findings are encouraging, although the role of moxifloxacin in clinical practice should be determined on the basis of its clinical outcomes.

CONCLUSION

The authors showed that 0.5% moxifloxacin was superior in ocular concentration than both 0.3% gatifloxacin and 0.5% levofloxacin. Moxifloxacin possesses excellent antibiotic activity against most pathogenic bacteria and superior ocular concentration, supporting its use in the prevention and treatment of ocular infections.

ACKNOWLEDGMENTS

This clinical study was funded by the Waksman Foundation of Japan (Tokyo, Japan). The authors thank all of the physicians and medical staff who assisted us in the study. They also thank Alcon Japan for supporting the publication of this manuscript.

Dr. Fukuda is the guarantor for this article, and takes responsibility for the integrity of the work as a whole.

Conflict of Interest. The authors have no conflict of interest.

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