7. Regillo CD, Brown DM, Abraham P, et al, PIER Study Group. Randomized, double-masked, sham-controlled trial of ranibizumab for neovascular age-related macular degeneration: PIER Study year 1. Am J Ophthalmol 2008;145:239–48.
8. Fung AE, Lalwani GA, Rosenfeld PJ, et al. An optical coherence tomography-guided, variable dosing regimen with intravitreal ranibizumab (Lucentis) for neovascular age-related macular degeneration. Am J Ophthalmol 2007;143:566–83.
9. Kokame GT, Yeung L, Lai JC. Continuous anti-VEGF treatment with ranibizumab for polypoidal choroidal vasculopathy: 6-month results. Br J Ophthalmol 2010;94:297–301.
10. Hikichi T, Ohtsuka H, Higuchi M, et al. Improvement of angiographic findings of polypoidal choroidal vasculopathy after intravitreal injection of ranibizumab monthly for 3 months. Am J Ophthalmol 2010;150:674–82.
11. Koizumi H, Yamagishi T, Yamazaki T, Kinoshita S. Predictive factors of resolved retinal fluid after intravitreal ranibizumab for polypoidal choroidal vasculopathy. Br J Ophthalmol 2011;95:1555–9.
12. Lai TY, Lee GK, Luk FO, Lam DS. Intravitreal ranibizumab with or without photodynamic therapy for the treatment of symptomatic polypoidal choroidal vasculopathy. Retina 2011; 31:1581–8.
13. Rouvas AA, Papakostas TD, Ntouraki A, et al. Photodynamic therapy, ranibizumab, and ranibizumab with photodynamic therapy for the treatment of polypoidal choroidal vasculopathy. Retina 2011;31:464–74.
14. Atmani K, Voigt M, Le Tien V, et al. Ranibizumab for retinal angiomatous proliferation in age-related macular degeneration. Eye (Lond) 2010;24:1193–8.
15. Engelbert M, Zweifel SA, Freund KB. "Treat and extend" dosing of intravitreal antivascular endothelial growth factor therapy for type 3 neovascularization/retinal angiomatous proliferation. Retina 2009;29:1424–31.
16. Hemeida TS, Keane PA, Dustin L, et al. Long-term visual and anatomical outcomes following anti-VEGF monotherapy for retinal angiomatous proliferation. Br J Ophthalmol 2010;94:701–5.
17. Rouvas AA, Papakostas TD, Vavvas D, et al. Intravitreal ranibizumab, intravitreal ranibizumab with PDT, and intravitreal triamcinolone with PDT for the treatment of retinal angiomatous proliferation: a prospective study. Retina 2009; 29:536–44.
18. Lai TY, Chan WM, Liu DT, Lam DS. Ranibizumab for retinal angiomatous proliferation in neovascular age-related macular degeneration. Graefes Arch Clin Exp Ophthalmol 2007;245: 1877–80.
19. Mori K, Gehlbach PL, Ito YN, Yoneya S. Decreased arterial dye-filling and venous dilation in the macular choroid associated with age-related macular degeneration. Retina 2005;25: 430–7.
20. Giovannini A, Mariotti C, Ripa E, et al. Choroidal filling in age-related macular degeneration: indocyanine green angiographic findings. Ophthalmologica 1994;208:185–91.
21. Lutty G, Grunwald J, Majji AB, et al. Changes in choriocapillaris and retinal pigment epithelium in age-related macular degeneration. Mol Vis [serial online] 1999;5:35. Available at: http://www.molvis.org/molvis/v5/a35/. Accessed February 13, 2012.
22. Pauleikhoff D, Spital G, Radermacher M, et al. A fluorescein and indocyanine green angiographic study of choriocapillaris in age-related macular disease. Arch Ophthalmol 1999;117:1353–8.
23. Koizumi H, Iida T, Saito M, et al. Choroidal circulatory disturbances associated with retinal angiomatous proliferation on indocyanine green angiography. Graefes Arch Clin Exp Ophthalmol 2008;246:515–20.
24. Schmidt-Erfurth U, Michels S, Barbazetto I, Laqua H. Photodynamic effects on choroidal neovascularization and physiological choroid. Invest Ophthalmol Vis Sci 2002;43:830–41.
25. Klais CM, Ober MD, Freund KB, et al. Choroidal infarction following photodynamic therapy with verteporfin. Arch Ophthalmol 2005;123:1149–53.
26. Spaide RF, Koizumi H, Pozonni MC. Enhanced depth imaging spectral-domain optical coherence tomography. Am J Ophthalmol 2008;146:496–500.
27. Margolis R, Spaide RF. A pilot study of enhanced depth imaging optical coherence tomography of the choroid in normal eyes. Am J Ophthalmol 2009;147:811–5.
28. Fujiwara T, Imamura Y, Margolis R, et al. Enhanced depth imaging optical coherence tomography of the choroid in highly myopic eyes. Am J Ophthalmol 2009;148:445–50.
29. Koizumi H, Yamagishi T, Yamazaki T, et al. Subfoveal choroidal thickness in typical age-related macular degeneration and polypoidal choroidal vasculopathy. Graefes Arch Clin Exp Ophthalmol 2011;249:1123–8.
30. Chung SE, Kang SW, Lee JH, Kim YT. Choroidal thickness in polypoidal choroidal vasculopathy and exudative age-related macular degeneration. Ophthalmology 2011;118:840–5.
31. Maruko I, Iida T, Sugano Y, et al. Subfoveal retinal and choroidal thickness after verteporfin photodynamic therapy for polypoidal choroidal vasculopathy. Am J Ophthalmol 2011;151:594–603.
32. Imamura Y, Fujiwara T, Margolis R, Spaide RF. Enhanced depth imaging optical coherence tomography of the choroid in central serous chorioretinopathy. Retina 2009;29:1469–73.
33. Maruko I, Iida T, Sugano Y, et al. Subfoveal choroidal thickness after treatment of Vogt-Koyanagi-Harada disease. Retina 2011;31:510–7.
34. Reibaldi M, Boscia F, Avitabile T, et al. Enhanced depth imaging optical coherence tomography of the choroid in idiopathic macular hole: a cross-sectional prospective study. Am J Ophthalmol 2011;151:112–7.
35. Imamura Y, Iida T, Maruko I, et al. Enhanced depth imaging optical coherence tomography of the sclera in dome-shaped macula. Am J Ophthalmol 2011;151:297–302.
36. Maul EA, Friedman DS, Chang DS, et al. Choroidal thickness measured by spectral domain optical coherence tomography: factors affecting thickness in glaucoma patients. Ophthalmology 2011;118:1571–9.
37. Mwanza JC, Hochberg JT, Banitt MR, et al. Lack of association between glaucoma and macular choroidal thickness measured with enhanced depth-imaging optical coherence tomography. Invest Ophthalmol Vis Sci 2011;52:3430–5.
38. Torres VL, Brugnoni N, Kaiser PK, Singh AD. Optical coherence tomography enhanced depth imaging of choroidal tumors. Am J Ophthalmol 2011;151:586–93.
39. Spaide RF, Yannuzzi LA, Slakter JS, et al. Indocyanine green videoangiography of idiopathic polypoidal choroidal vasculopathy. Retina 1995;15:100–10.
40. Yannuzzi LA, Negrao S, Iida T, et al. Retinal angiomatous proliferation in age-related macular degeneration. Retina 2001; 21:416–34.
41. Funk M, Karl D, Georgopoulos M, et al. Neovascular age-related macular degeneration: intraocular cytokines and growth factors and the influence of therapy with ranibizumab. Ophthalmology 2009;116:2393–9.
42. Gaudreault J, Fei D, Beyer JC, et al. Pharmacokinetics and retinal distribution of ranibizumab, a humanized antibody fragment directed against VEGF-A, following intravitreal administration in rabbits. Retina 2007;27:1260–6.
43. Lim SJ, Roh MI, Kwon OW. Intravitreal bevacizumab injection for central serous chorioretinopathy. Retina 2010;30:100–6.

44. Papadopoulou DN, Mendrinos E, Mangioris G, et al. Intravitreal ranibizumab may induce retinal arteriolar vasoconstriction in patients with neovascular age-related macular degeneration. Ophthalmology 2009;116:1755–61.

45. Sacu S, Pemp B, Weigert G, et al. Response of retinal vessels and retrobulbar hemodynamics to intravitreal anti-VEGF treatment in eyes with branch retinal vein occlusion. Invest Ophthalmol Vis Sci 2011;52:3046–50.

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ORIGINAL RESEARCH

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

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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

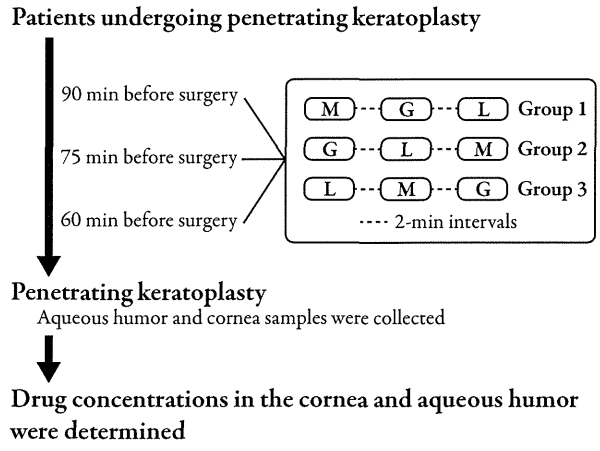

Fig. 1 Study protocol. *G* gatifloxacin, *L* levofloxacin, *M* moxifloxacin, *min* minutes

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

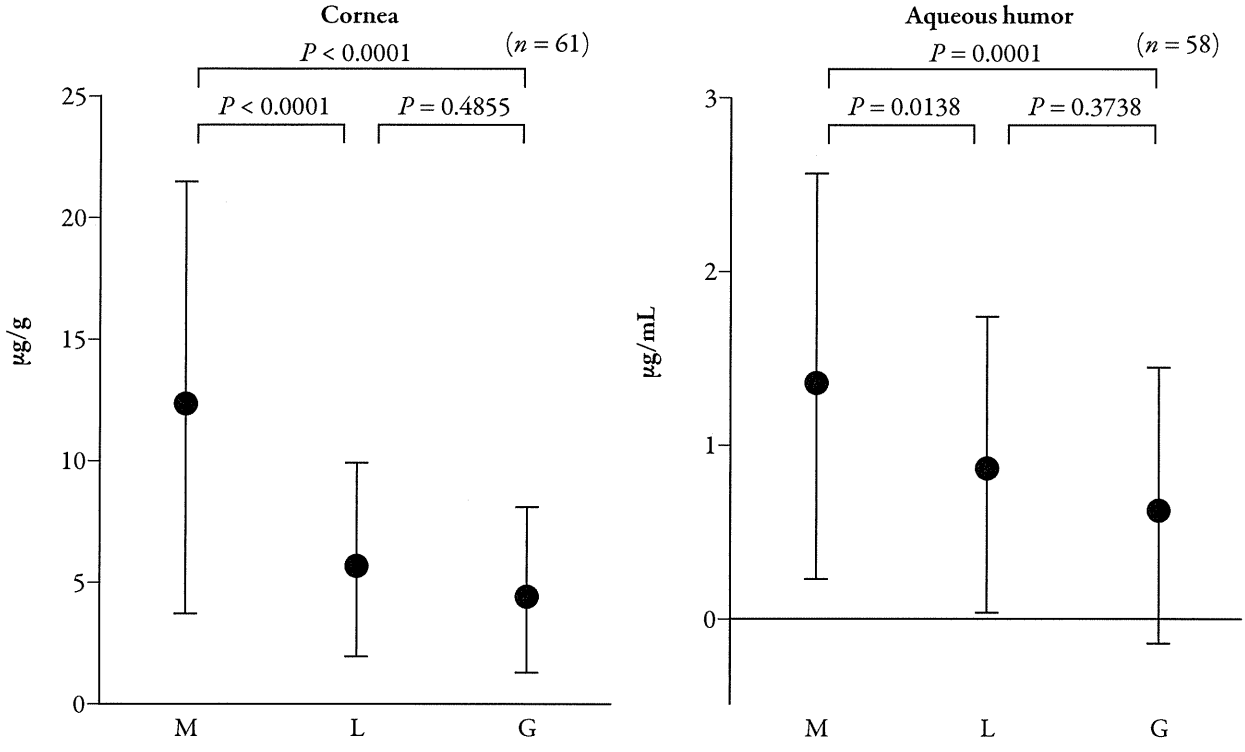

Fig. 2 Corneal and aqueous humor concentrations (mean ± SD) of the three fluoroquinolones studied. Statistical comparisons were made using Tukey's multiple comparison test. *G* gatifloxacin, *L* levofloxacin, *M* moxifloxacin

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.

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

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REFERENCES

1. Miller JJ, Scott IU, Flynn HW Jr, Smiddy WE, Newton J, Miller D. Acute-onset endophthalmitis after cataract surgery (2000–2004): incidence, clinical settings, and visual acuity outcomes after treatment. Am J Ophthalmol. 2005;139:983–87.
2. Kowalski RP, Dhaliwal DK, Karenchak LM, et al. Gatifloxacin and moxifloxacin: an in vitro susceptibility comparison to levofloxacin, ciprofloxacin, and ofloxacin using bacterial keratitis isolates. Am J Ophthalmol. 2003;136:500–5.
3. Mather R, Karenchak LM, Romanowski EG, Kowalski RP. Fourth generation fluoroquinolones: new weapons in the arsenal of ophthalmic antibiotics. Am J Ophthalmol. 2002;133:463–6.
4. Scoper SV. Review of third-and fourth-generation fluoroquinolones in ophthalmology: in-vitro and in-vivo efficacy. Adv Ther. 2008;25:979–94.
5. Mauger TF. Antimicrobials. In: Mauger TF, Craig EL, eds. Havener's Ocular Pharmacology. 6th edition. St. Louis: Mosby; 1994:234–349.
6. Fukuda M, Sasaki H. Calculation of AQCmax: comparison of five ophthalmic fluoroquinolone solutions. Curr Med Res Opin. 2008;24:3479–85.
7. Sugioka K, Fukuda M, Komoto S, Itahashi M, Yamada M, Shimomura Y. Intraocular penetration of sequentially instilled topical moxifloxacin, gatifloxacin, and levofloxacin. Clin Ophthalmol. 2009;3:553–7.
8. Kim DH, Stark WJ, O'Brien TP, Dick JD. Aqueous penetration and biological activity of moxifloxacin 0.5% ophthalmic solution and gatifloxacin 0.3% solution in cataract surgery patients. Ophthalmology. 2005;112:1992–6.
9. McCulley JP, Caudle D, Aronowicz JD, Sine WE. Fourth-generation fluoroquinolone penetration into the aqueous humor in humans. Ophthalmology. 2006;113:955–9.
10. Holland EJ, Lane SS, Kim T, Raizman M, Dunn S. Ocular penetration and pharmacokinetics of topical gatifloxacin 0.3% and moxifloxacin 0.5% ophthalmic solutions after keratoplasty. Cornea. 2008;27:314–9.
11. Owen GR, Brooks AC, James O, Robertson SM. A novel in vivo rabbit model that mimics human dosing to determine the distribution of antibiotics in ocular tissues. J Ocul Pharmacol Ther. 2007;23:335–42.

12. Yamada M, Mochizuki H, Yamada K, Kawai M, Mashima Y. Aqueous humor levels of topically applied levofloxacin, norfloxacin, and lomefloxacin in the same human eyes. J Cataract Refract Surg. 2003;29:1771–5.

13. Yamada M, Ishikawa K, Mochizuki H, Kawai M. Corneal penetration of simultaneously applied topical levofloxacin, norfloxacin and lomefloxacin in human eyes. Acta Ophthalmol Scand. 2006;84:192–6.

14. Liesegang TJ. Use of antimicrobials to prevent postoperative infection in patients with cataracts. Curr Opin Ophthalmol. 2001;12:68–74.

15. Katz HR, Masket S, Lane SS, et al. Absorption of topical moxifloxacin ophthalmic solution into human aqueous humor. Cornea.2005:24:955-8.

16. Robertson SM, Curtis MA, Schlech BA, et al. Ocular pharmacokinetics of moxifloxacin after topical treatment of animals and humans. Surv Ophthalmol. 2005;50:S32–S45.

17. Baudouin C. Detrimental effect of preservatives in eyedrops: implications for the treatment of glaucoma. Acta Ophthalmol. 2003;136:76–81.

18. Hyon JY, Eser I, O'Brien TP. Kill rates of preserved and preservative-free topical 8-methoxy fluoroquinolones against various strains of Staphylococcus. J Cataract Refract Surg. 2009;35:1609–13.

19. Donnenfeld ED, Perry HD, Snyder RW, Moadel R, Elsky M, Jones H. Intracorneal, aqueous humor, and vitreous humor penetration of topical and oral ofloxacin. Arch Ophthalmol. 1997;115:173–6.

20. Price FW Jr, Whitson WE, Gonzales J, Johns S. Corneal tissue levels of topically applied ofloxacin. J Cataract Refract Surg. 1997;23:898–902.

21. Diamond JP, White L, Leeming JP, Bing Hoh H, Easty DL. Topical 0.3% ciprofloxacin, norfloxacin, and ofloxacin in treatment of bacterial keratitis: a new method for comparative evaluation of ocular drug penetration. Br J Ophthalmol. 1995;79:606–9.

22. DeSantis LM. Pharmacokinetics. In: Mauger TF, Craig EL, eds. Havener's Ocular Pharmacology. 6th edition. St. Louis: Mosby; 1994:22–52.

23. Colleaux KM, Hamilton WK. Effect of prophylactic antibiotics and incision type on the incidence of endophthalmitis after cataract surgery. Can J Ophthalmol. 2000;35:373–8.

24. Brown L. Resistance to ocular antibiotics: an overview. Clin Exp Optom. 2007;90:258–62.

25. Hwang DG. Fluoroquinolone resistance in ophthalmology and the potential role for newer ophthalmic fluoroquinolones. Surv Ophthalmol. 2004;49:S79–83.

26. Alexandrakis G, Alfonso EC, Miller D. Shifting trends in bacterial keratitis in south Florida and emerging resistance to fluoroquinolones. Ophthalmology. 2000;107:1497–502.

27. Goldstein MH, Kowalski RP, Gordon YJ. Emerging fluoroquinolone resistance in bacterial keratitis: a 5-year review. Ophthalmology. 1999;106: 1313–18.

28. Firsov AA, Vostrov SN, Lubenko IY, Drlica K, Portnoy YA, Zinner SH. In vitro pharmacodynamic evaluation of the mutant selection window hypothesis using four fluoroquinolones against Staphylococcus aureus. Antimicrob Agents Chemother. 2003;47:1604–13.

29. Zhao X, Drlica K. Restricting the selection of antibiotic-resistant mutant bacteria: measurement and potential use of the mutant selection window. J Infect Dis. 2002;185:561–5.

30. Kim DH, Stark WJ, O'Brien TP. Ocular penetration of moxifloxacin 0.5% and gatifloxacin 0.3% ophthalmic solutions into the aqueous humor following topical administration prior to routine cataract surgery. Curr Med Res Opin. 2005;21:93–4.

Fundus Autofluorescence in Polypoidal Choroidal Vasculopathy

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Purpose: To investigate and compare the characteristics of fundus autofluorescence (FAF) in polypoidal choroidal vasculopathy (PCV) with those in typical neovascular age-related macular degeneration (AMD).

Design: Retrospective, observational, consecutive case series.

Participants: Ninety-two patients with PCV (92 affected eyes and 86 unaffected fellow eyes) and 31 patients with typical neovascular occult AMD with no classic choroidal neovascularization (31 affected eyes and 24 unaffected fellow eyes).

Methods: All study eyes underwent FAF photography with a fundus camera–based system. The incidence and distribution of hypoautofluorescence, that is, the manifestation of retinal pigment epithelium (RPE) damages, were evaluated.

Main Outcome Measures: The characteristic FAF findings in PCV.

Results: In the affected eyes with PCV, the sites of the neovascular lesions showed 2 distinct FAF patterns: (1) the confluent hypoautofluorescence at the polypoidal lesions and (2) the granular hypoautofluorescence at the branching choroidal vascular networks. The confluent hypoautofluorescence, most of which was surrounded by a hyperautofluorescent ring, was seen in 74 eyes (80.4%) with PCV but was seen in no eyes with typical neovascular AMD ($P < 0.001$). The granular hypoautofluorescence was seen in 91 eyes (98.9%) with PCV and 27 eyes (87.1%) with typical neovascular AMD ($P = 0.014$). In addition, the eyes with PCV more frequently showed hypoautofluorescence outside the macular area than those with typical neovascular AMD ($P = 0.021$). In the unaffected fellow eyes, the hypoautofluorescence was more frequently observed in patients with PCV than in those with typical neovascular AMD, inside the macular area and in the entire FAF image ($P = 0.012$, $P = 0.003$, respectively).

Conclusions: In eyes with PCV, the polypoidal lesions and the branching choroidal vascular networks appeared to affect the RPE and induce peculiar FAF findings. When compared with the patients with typical neovascular AMD, widespread RPE damage was more frequently observed in the patients with PCV, both in the affected eyes and in the unaffected fellow eyes.

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Neovascular age-related macular degeneration (AMD) is one of the leading causes of legal blindness in developed and developing countries.[1] However, the clinical characteristics of neovascular AMD are diverse and vary among populations. Polypoidal choroidal vasculopathy (PCV) is generally considered to be a subtype of neovascular AMD and is characterized by peculiar orange-reddish polypoidal lesions beneath the retinal pigment epithelium (RPE).[2] Other clinical manifestations of PCV include multiple, recurrent serosanguineous detachments of the RPE and neurosensory retina secondary to leakage and bleeding from the neovascular lesions. Indocyanine green angiography (ICGA) allows for the visualization of the branching choroidal vascular networks that terminate in polypoidal dilations, and these 2 components are known to be characteristic findings in PCV.[3] Polypoidal choroidal vasculopathy is common in the Asian population compared with the Caucasian population.[4–9] Although the precise pathogenesis of PCV remains controversial, it is important to clinically differentiate between PCV and typical neovascular AMD, because these 2 entities reportedly show different natural courses and responses to treatments.[10]

Fundus autofluorescence (FAF) photography is used to visualize lipofuscin, which accumulates in RPE and provides information about RPE metabolism and function. Previous reports on FAF findings in neovascular AMD[11–13] have provided information regarding the RPE integrity in relation to choroidal neovascularization (CNV). However, and to the best of our knowledge, no previous report has focused specifically on the FAF findings in PCV. The current study investigated and compared the characteristics of FAF in PCV with those in typical neovascular AMD to gain new insights into the pathologic differences between these 2 disease entities.

Patients and Methods

In the current study, FAF images of consecutive Japanese patients with newly diagnosed PCV who were seen in the outpatient clinic for macular diseases at Kyoto Prefectural University of Medicine between December 2008 and February 2011 were retrospectively reviewed. For the purpose of comparison, the FAF images of the patients with typical neovascular occult AMD with no classic CNV seen during the same period were also evaluated. Classic CNV was found to be mainly composed of subretinal fibrous

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tissue,[14] resulting in possible blocked autofluorescence, rather than the signs of RPE abnormalities.[12] Therefore, the eyes diagnosed as having classic CNV on fluorescein angiography (FA) were excluded from this study. In addition to the FAF photography, all patients underwent comprehensive ocular examinations including refraction testing, best-corrected visual acuity (BCVA) testing using Landolt C charts, examination by slit-lamp biomicroscopy with contact or noncontact lenses, color fundus photography, FA, ICGA, and optical coherence tomography (OCT) at the time of the initial diagnosis.

Each diagnosis of PCV or typical neovascular AMD was based on the funduscopic and angiographic findings by a researcher (H.K.). The diagnosis of PCV was based on ICGA, which demonstrates polypoidal structures at the border of the branching choroidal vascular networks.[3] In some cases, subpigment epithelial orange-red protrusions were seen biomicroscopically, corresponding to the polypoidal lesions on ICGA. Typical neovascular AMD was characterized by exudative changes, and consistent CNV was revealed by FA and ICGA.

When the experienced physician was unable to establish the definitive diagnosis of PCV or typical neovascular AMD, such confusing cases were excluded from this study. If a patient had bilateral active neovascular lesions, the right eye of that patient was chosen as the study eye. For the evaluation of FAF images, eyes with scar formation secondary to the neovascular lesions were excluded. Eyes with massive subretinal hemorrhage or subpigment epithelial hemorrhage that obscured the FAF findings at the neovascular lesions were also excluded. Eyes with a spherical equivalent of −6 diopters or less or chorioretinal atrophic changes secondary to pathologic myopia and patients with other macular disorders, such as angioid streaks, central serous chorioretinopathy (CSC), and retinal angiomatous proliferation, were also excluded.

Color fundus photography and FAF photography were performed with a commercially available fundus camera system (TRC-50DX, Topcon Corp, Tokyo, Japan) with a 50-degree field of view. In the FAF mode, the device used blue light with an excitation filter centered at 560 nm (bandwidth, 535–585 nm) and a barrier filter centered at 655 nm (bandwidth, 605–705 nm). The fovea was centrally located in all FAF images. Fluorescein angiography and ICGA were performed with a confocal scanning laser ophthalmoscope (Heidelberg Retina Angiograph 2, Heidelberg Engineering, Heidelberg, Germany). The cross-sectional images of the macular area were obtained by spectral-domain OCT (3D-OCT 1000 Mark II, Topcon Corp).

In our review of the published literature, we conducted a search of MEDLINE with PubMed. Search words included *polypoidal choroidal vasculopathy*, *PCV,* and *autofluorescence*, and the results of our search informed us that the FAF findings in PCV had not been reported. In this study, 2 researchers (T.Y. and T.Y.) who were unfamiliar with the patients' biomicroscopic and angiographic information specifically evaluated the incidence and distribution of hypoautofluorescence (i.e., the manifestations of RPE damage at various sites). With regard to the affected eyes, the incidence of hypoautofluorescence at the neovascular lesions and outside the macular area was investigated. For the unaffected fellow eyes, the incidence of hypoautofluorescence inside and outside the macular area was also assessed. The hypoautofluorescence was classified into the following 2 patterns: (1) confluent hypoautofluorescence and (2) granular hypoautofluorescence. The confluent hypoautofluorescence was defined as a manifestation of a homogeneous lack of autofluorescence that was well demarcated and clearly distinguishable from the other adjacent lesions. The granular hypoautofluorescence was defined as a heterogeneous mixed finding of the hypoautofluorescence lesion at the various levels. Blocked autofluorescence induced by other causes, such as subretinal hemorrhages, was not considered as a manifestation of RPE damage. The macular area was defined as a 6.0-mm diameter area around the foveola.

The obtained data were analyzed as to the frequency and descriptive statistics. The BCVA measurements were converted to logarithm of the minimum angle of resolution units before analysis. Chi-square testing was used for categoric analysis. The Fisher exact test was used if the expected cell count was less than 5. Statistical analyses for the nonparametric data used the Mann–Whitney *U* test. All statistical analyses were performed with StatView software version 5.0 (SAS Inc, Cary, NC). A *P* value less than 0.05 was considered statistically significant for all tests, and all tests were 2-sided. 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.

Table 1. Demographic Features of Patients with Polypoidal Choroidal Vasculopathy and Typical Neovascular Age-Related Macular Degeneration

	PCV Group (n = 92)	AMD Group (n = 31)	*P* Value
Female (%)	25 (27.2%)	8 (29.0%)	1.00
Age (yrs) ± SD	74.6±7.6	75.4±10.0	0.65
Mean logMAR BCVA ± SD in the affected eyes	0.52±0.39	0.53±0.31	0.89

AMD = age-related macular degeneration; BCVA = best-corrected visual acuity; logMAR = logarithm of minimum angle of resolution; PCV = polypoidal choroidal vasculopathy; SD = standard deviation.

Table 2. Hypoautofluorescent Findings in the Affected Eyes

	PCV Group (n = 92)	AMD Group (n = 31)	*P* Value
Confluent hypoautofluorescence at the neovascular lesion (%)	74 (80.4%)	0 (0%)	<0.0001*
Granular hypoautofluorescence at the neovascular lesion (%)	91 (98.9%)	27 (87.1%)	0.014†
Hypoautofluorescence outside the macular area (%)	39 (42.4%)	6 (19.4%)	0.021†

AMD = age-related macular degeneration; PCV = polypoidal choroidal vasculopathy.
*$P < 0.01$; †$P < 0.05$.

Table 3. Hypoautofluorescent Findings in the Unaffected Fellow Eyes

	PCV Group (n = 86)	AMD Group (n = 24)	*P* Value
Hypoautofluorescence in the entire image (%)	54 (62.8%)	7 (29.2%)	0.003*
Hypoautofluorescence inside the macular area (%)	50 (58.1%)	7 (29.2%)	0.012†
Hypoautofluorescence outside the macular area (%)	26 (30.2%)	3 (12.5%)	0.081

AMD = age-related macular degeneration; PCV = polypoidal choroidal vasculopathy.
*$P < 0.01$.
†$P < 0.05$.

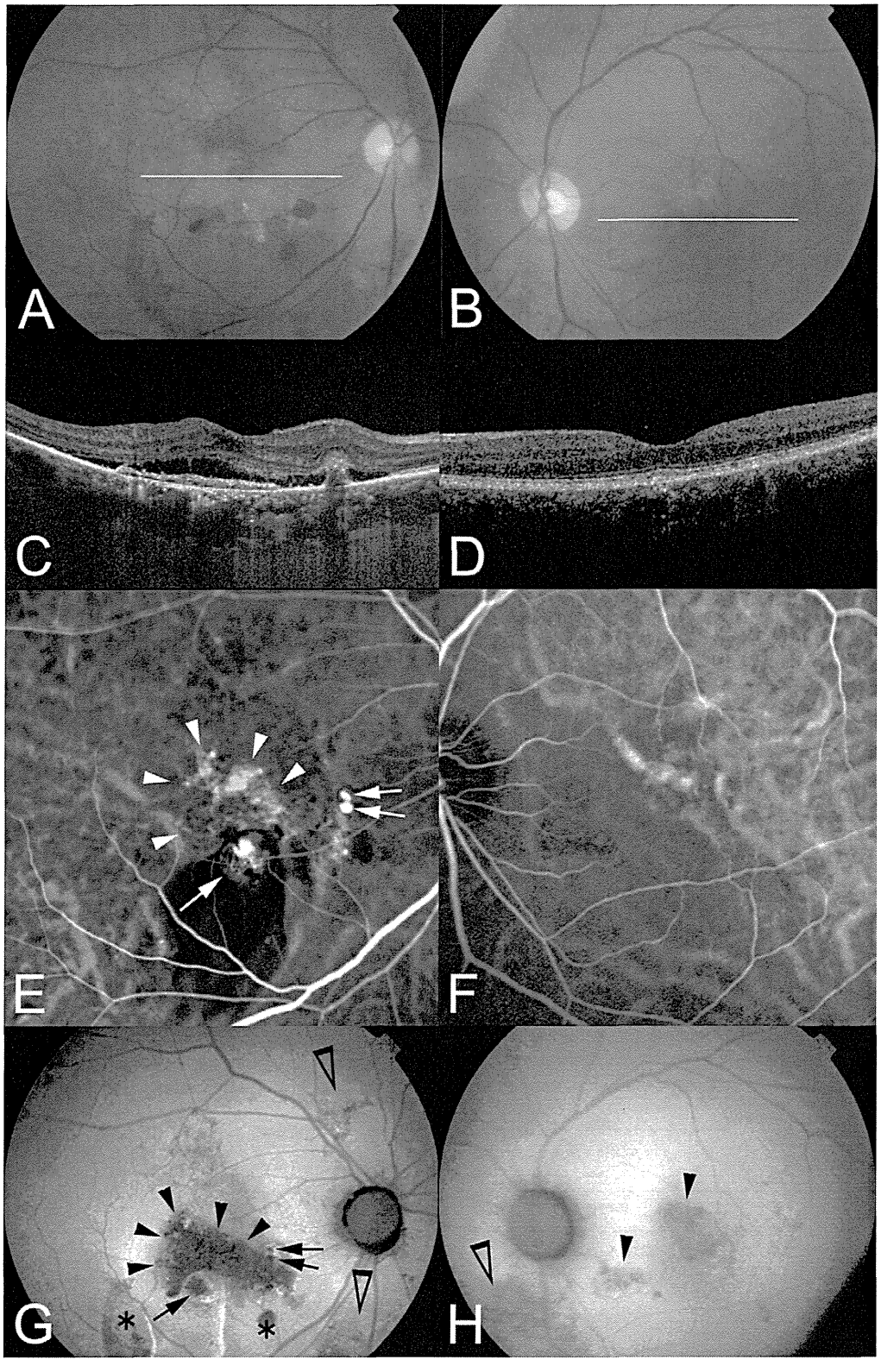

Figure 1.

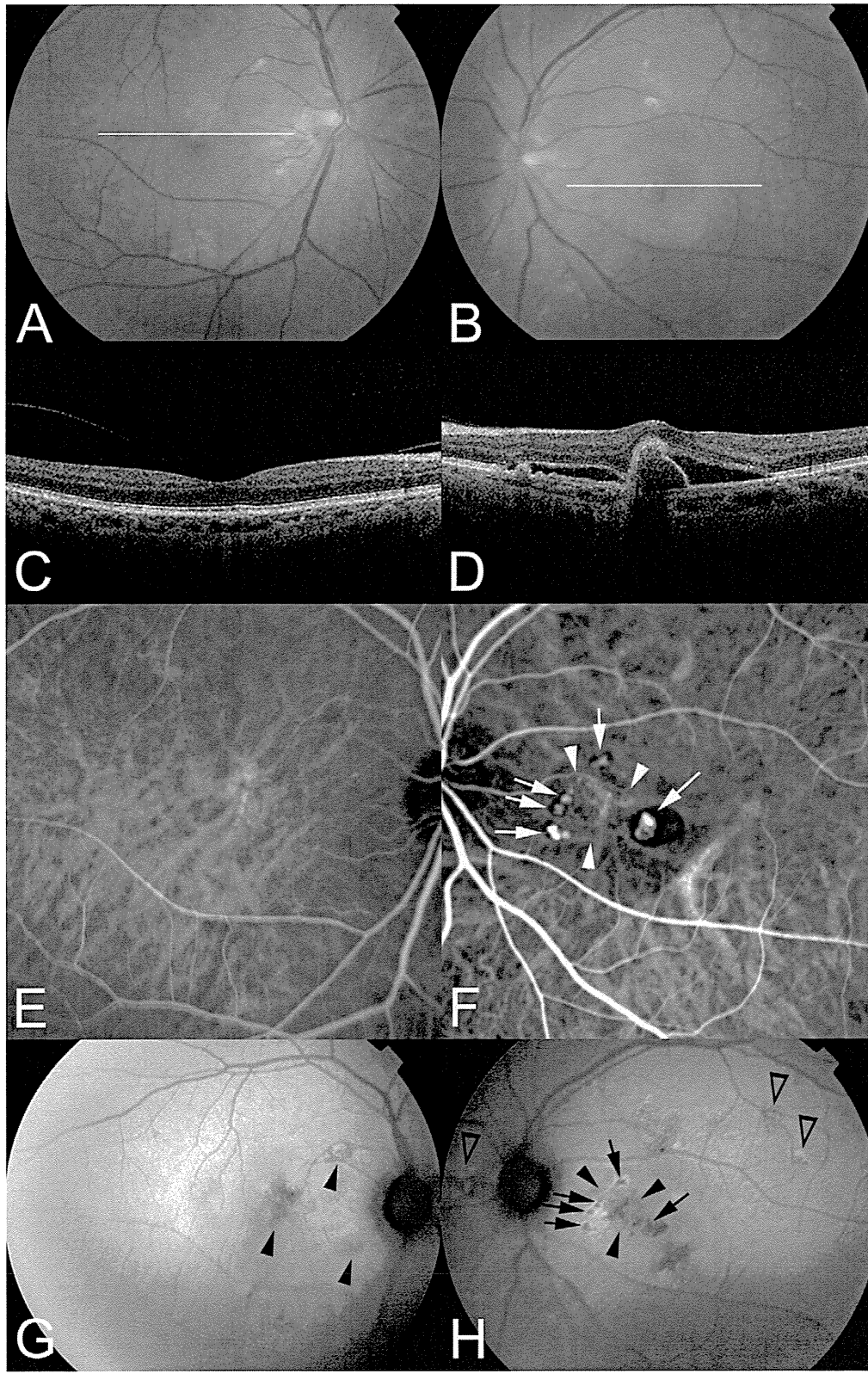

Figure 2.

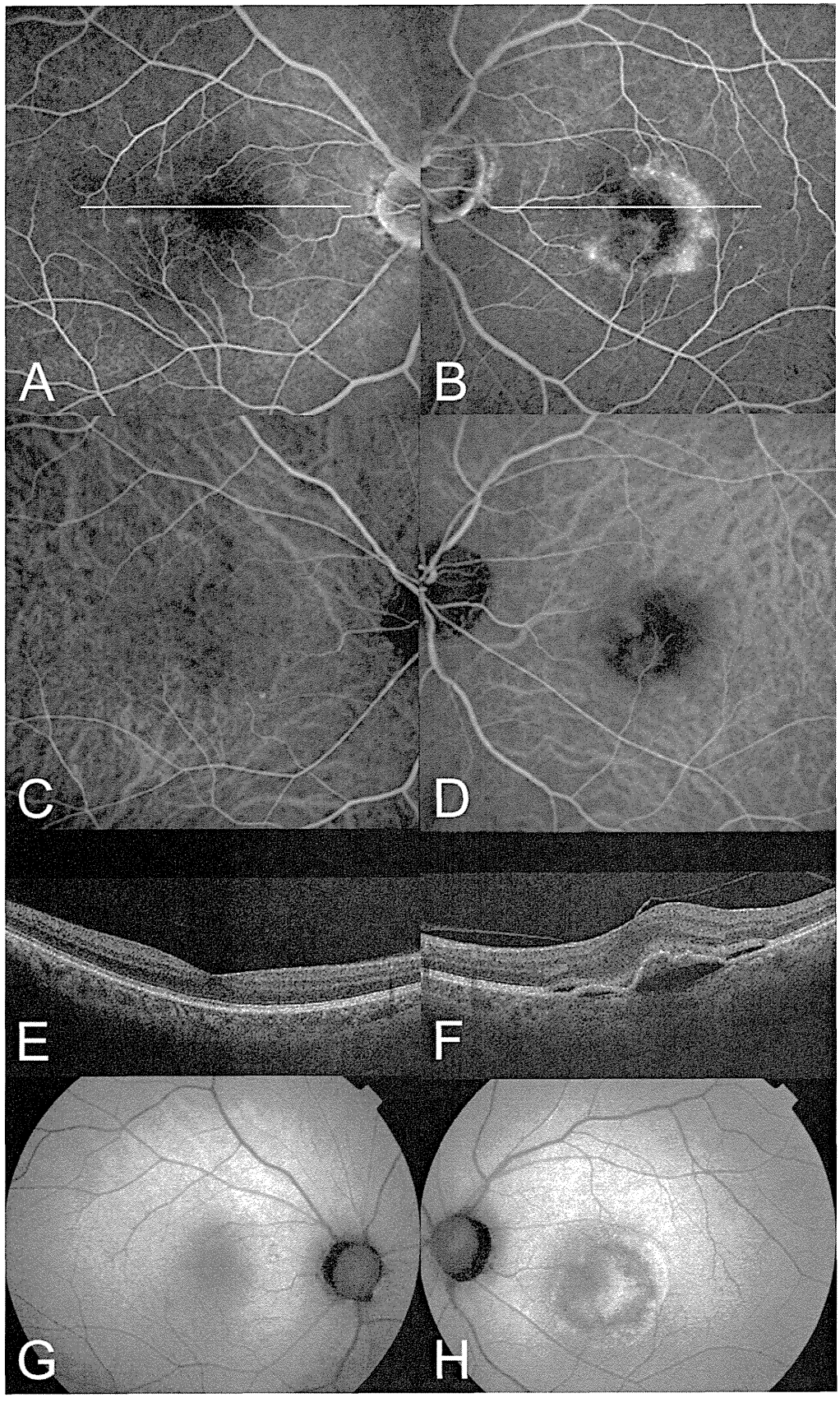

Figure 3.

Results

Ninety-two patients with PCV (PCV group) and 31 patients with typical neovascular occult AMD with no classic CNV (AMD group) matched the inclusion criteria. There were no significant differences in gender, age, or BCVA in the affected eyes between the PCV group and the AMD group (Table 1).

Of the 92 patients in the PCV group, 6 patients had bilateral neovascular lesions. Of those 6 patients, 5 showed scar formation in the contralateral eye, and those 5 eyes were excluded from the FAF image evaluations. The other patient had active PCV lesions bilaterally, and the right eye was selected as the affected eye. Therefore, 92 eyes were evaluated as the affected eyes, and 86 eyes were evaluated as the unaffected fellow eyes. Of the 92 affected eyes, the neovascular lesion was located at the subfovea in 63 eyes, at the juxtafovea in 16 eyes, at the extrafovea in 7 eyes, and in the peripapillary area in 6 eyes.

Of the 31 patients in the AMD group, 7 patients had bilateral CNV; however, all 7 patients showed fibrotic scar formation in the contralateral eye. Accordingly, 31 eyes were evaluated as the affected eyes, and 24 eyes were evaluated as the unaffected fellow eyes. Of the 31 affected eyes, the CNV was located at the subfovea in 25 eyes, at the juxtafovea in 4 eyes, and at the extrafovea in 2 eyes.

The FAF findings in the affected eyes are shown in Table 2. At the sites of the neovascular lesions, confluent hypoautofluorescence (termed as "punched-out lesion" in this study) was observed in 80.4% of the eyes in the PCV group, yet was not observed in any of the eyes in the AMD group ($P < 0.001$). All of the confluent hypoautofluorescence observed in the PCV group corresponded to polypoidal lesions seen on ICGA. In addition, most of the confluent hypoautofluorescence was surrounded by a hyperautofluorescent ring. The granular hypoautofluorescence was observed in most of the PCV group (98.9%) at the branching choroidal vascular networks and in the AMD group (87.1%) at the CNV, yet it was significantly more frequently observed in the PCV group ($P = 0.014$). The hypoautofluorescence outside the macular area was significantly more frequently observed in the PCV group (42.4%) than in the AMD group (19.4%) ($P = 0.021$). All of the hypoautofluorescence observed outside the macular area appeared to be granular hypoautofluorescence.

The FAF findings in the unaffected fellow eyes are shown in Table 3. The hypoautofluorescence inside the macular area and in the entire FAF image was significantly more frequently observed in the PCV group than in the AMD group (58.1% vs 29.2% [$P = 0.012$] and 62.6% vs 29.2% [$P = 0.003$], respectively). The hypoautofluorescence outside the macular area was more frequently seen in the PCV group than in the AMD group, but the difference did not reach a significant level ($P = 0.081$). All of the hypoautofluorescence in the unaffected fellow eyes was found to be granular hypoautofluorescence. The representative cases are shown in Figures 1 to 3.

Discussion

The results of this study demonstrated the characteristic FAF findings of PCV in comparison with those of typical neovascular AMD. The PCV-associated neovascular lesions exhibited the combination of 2 distinct FAF patterns: confluent hypoautofluorescence and granular hypoautofluores-

Figure 1. A 75-year-old man with polypoidal choroidal vasculopathy (PCV). Biomicroscopic examination of the right eye **(A)** revealed irregular elevation of retinal pigment epithelium (RPE) with orange-reddish nodules, accompanied by multiple areas of subretinal hemorrhages. The left eye **(B)** showed mild RPE depigmentation in the macula. Optical coherence tomography along the white lines **(A, B)** showed subretinal fluid accumulation and irregularly elevated RPE in the right eye **(C)** and no exudative changes in the left eye **(D)**. Indocyanine green angiography of the right eye **(E)** revealed characteristic components of PCV consisting of the polypoidal structures (*arrows*) and the branching choroidal vascular networks (*arrowheads*), but no neovascular lesion was seen in the left eye **(F)**. The fundus autofluorescence (FAF) imaging of the right eye **(G)** demonstrated confluent hypoautofluorescence ("punched-out lesion") surrounded by hyperautofluorescent rings (*arrows*) at the polypoidal lesions seen on indocyanine green angiography (ICGA) and granular hypoautofluorescence at the branching choroidal vascular network (*arrowheads*). In addition, the granular hypoautofluorescence was also seen outside the macular area (*open arrowheads*). *Asterisks* indicate blocked autofluorescence induced by the subretinal hemorrhages. The FAF image of the left eye **(H)** showed granular hypoautofluorescence inside (*arrowheads*) and outside (*open arrowhead*) the macular area.

Figure 2. A 70-year-old man with polypoidal choroidal vasculopathy (PCV). Biomicroscopic examination demonstrated no notable finding in the right eye **(A)** and an orange-reddish lesion at the fovea in the left eye **(B)**. Optical coherence tomography along the white lines **(A, B)** revealed no exudative findings in the right eye **(C)** and a high protrusion of the retinal pigment epithelium (RPE) at the fovea accompanied by subretinal fluid accumulation in the left eye **(D)**. Indocyanine green angiography (ICGA) of the right eye **(E)** showed no neovascular lesion, whereas there were several polypoidal lesions (*arrows*) connected to the branching choroidal neovascular networks (*arrowheads*) in the left eye **(F)**. Fundus autofluorescence photography of the right eye **(G)** showed multifocal areas of the granular hypoautofluorescence inside the macular area (*arrowheads*). The left eye **(H)** showed the confluent hypoautofluorescence ("punched-out lesion") surrounded by hyperautofluorescent rings (*arrows*) at the polypoidal lesions seen on ICGA and the granular hypoautofluorescence at the branching choroidal vascular networks (*arrowheads*). Note that the granular hypoautofluorescence was also present outside the macular area (*open arrowheads*).

Figure 3. A 61-year-old man with typical neovascular age-related macular degeneration (AMD). Fluorescein angiography of the right eye **(A)** showed no sign of neovascular AMD, whereas the left eye **(B)** showed occult AMD with no classic choroidal neovascularization (CNV). Indocyanine green angiography of the right eye **(C)** demonstrated no particular abnormalities, but the left eye **(D)** appeared to show the irregular hyperfluorescence suggestive of CNV in the macula. Optical coherence tomography along the white lines **(A, B)** in the right eye **(E)** was normal, but the left eye **(F)** showed some hyperreflectivity beneath the irregular pigment epithelial detachment accompanied by subretinal fluid accumulation. Fundus autofluorescence (FAF) photography of the patient's right eye **(G)** demonstrated a normal FAF finding, whereas that of the left eye **(H)** showed continuous autofluorescence in the macular area without evident hypoautofluorescence.

cence. The former was represented by well-demarcated areas of hypoautofluorescence, most of which were surrounded by a hyperautofluorescent ring, corresponding to the polypoidal lesions seen on ICGA images. This characteristic "punched-out lesion" pattern was observed in most of the eyes with PCV, yet not observed in the eyes with typical neovascular AMD. Thus, the confluent hypoautofluorescence appeared to be peculiar to PCV, thus indicating that the polypoidal lesions might induce significant damage to the RPE layers. Prominent anterior protrusions at the sites of the polypoidal lesions were observed by use of OCT,[15] and those protrusions may cause marked mechanical stress on the overlying RPE layer.

The sites of the branching choroidal vascular networks in PCV exhibited granular hypoautofluorescence in all but 1 eye, somewhat more frequently than those of CNV in typical neovascular AMD. This finding suggests that the RPE layer might be affected not only by the polypoidal lesions but also by the branching choroidal vascular networks. In previous reports, there have been controversies with regard to the localization of the branching choroidal vascular networks. In some studies, the authors considered that they are located in the sub-RPE space, similar to CNV seen in typical neovascular AMD,[16] whereas in other studies, it was theorized that they are in the choroid.[17] The FAF manifestation might be consistent with the findings shown on spectral-domain OCT images in which the branching choroidal vascular networks, as well as the polypoidal lesions, exist between the RPE and the Bruch's membrane and adhere to the back surface of the RPE layers.[18] By using a time-domain OCT, Sato et al[19] reported about the "double layer sign" corresponding to the branching choroidal vascular networks and demonstrated the fluid accumulation between the RPE and the Bruch's membrane. Similar to the polypoidal lesions, the branching choroidal vascular networks seem to produce an exudative property and may produce a significant hemodynamic effect on the RPE layer.

In comparison with typical neovascular AMD, the hypoautofluorescence in the macular area of the unaffected fellow eyes in this study was found to be more frequent in PCV. Ueta et al[20] reported that RPE atrophy in the macular area was the prevailing feature of the unaffected fellow eyes of patients with PCV, consistent with the findings of this present study. Moreover, in patients with PCV, the hypoautofluorescence outside the macular area in the affected eyes and, although not statistically significant, outside the macular area in the unaffected fellow eyes was more frequently observed than in patients with typical neovascular AMD. Such FAF findings might suggest that PCV causes more widespread RPE damage than typical neovascular AMD. Sasahara et al[21] reported that ICGA demonstrated the findings of bilateral and multifocal choroidal vascular hyperpermeability more frequently in PCV than in typical neovascular AMD. Thus, subclinical stress induced by the hemodynamic changes in the choroid might be put on the RPE layer, resulting in the widespread hypoautofluorescence in eyes with PCV, and even in the contralateral eyes. Similar widespread RPE changes, shown by hypoautofluorescent findings, are also known to appear in CSC,[22] and might suggest, at least in part, a common pathologic background between PCV and CSC.

It still remains controversial as to whether PCV represents a subtype of neovascular AMD.[10] However, the clinical discrimination between PCV and typical neovascular AMD is important, especially when considering the optimal treatment strategy, such as intravitreal injection of antivascular endothelial growth factor agents and photodynamic therapy. The FAF findings shown in this current study, and as an adjunct to the other conventional imaging methods, may prove valuable for supporting more definite diagnoses of PCV or typical neovascular AMD. Furthermore, in future epidemiologic research, noninvasive and accurate classification of the subtypes of neovascular AMD might be possible by means of FAF photography, for example, in conjunction with higher-resolution OCT without FA and ICGA.

Study Limitations

The current study has major limitations inherent in any retrospective study and the limited number of cases. In addition, this study was only a cross-sectional study, and the longitudinal changes of FAF over time in a subject should be investigated in future studies. In this study, the evaluation of FAF images was qualitative, not quantitative. Furthermore, this study did not take into consideration the duration of symptoms in each patient, which might have an effect on the FAF findings.

In conclusion, despite these limitations, and to the best of our knowledge, this study is the first to show the characteristics of FAF specific to PCV eyes and might shed some light on the pathophysiology. Both the polypoidal lesions and the branching choroidal vascular networks appeared to affect the RPE and resulted in peculiar FAF findings. In comparison with typical neovascular AMD, the subclinical and widespread RPE damages were more frequent in PCV, both in the affected eyes and the unaffected fellow eyes. Fundus autofluorescence imaging might prove to be helpful and clinically beneficial as an adjunct tool in the diagnosis and management of PCV.

References

1. Wong TY, Chakravarthy U, Klein R, et al. The natural history and prognosis of neovascular age-related macular degeneration: a systematic review of the literature and meta-analysis. Ophthalmology 2008;115:116–26.
2. Yannuzzi LA, Sorenson J, Spaide RF, Lipson B. Idiopathic polypoidal choroidal vasculopathy (IPCV). Retina 1990; 10:1–8.
3. Spaide RF, Yannuzzi LA, Slakter JS, et al. Indocyanine green videoangiography of idiopathic polypoidal choroidal vasculopathy. Retina 1995;15:100–10.
4. Sho K, Takahashi K, Yamada H, et al. Polypoidal choroidal vasculopathy: incidence, demographic features, and clinical characteristics. Arch Ophthalmol 2003;121:1392–6.
5. Wen F, Chen C, Wu D, Li H. Polypoidal choroidal vasculopathy in elderly Chinese patients. Graefes Arch Clin Exp Ophthalmol 2004;242:625–9.