Frequency Doubling Technology and Scanning Laser Tomography in Eyes With Generalized Enlargement of Optic Disc Cupping

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Purpose: To characterize functional and structural changes in eyes with generalized enlargement of optic disc cupping (vertical cup/disc ratio ≥0.8), normal intraocular pressure, normal standard achromatic automated perimetry (SAP) results, and no other ophthalmoscopic findings suggesting glaucoma (large C/D eyes) using frequency doubling technology (FDT) and the Heidelberg Retina Tomograph (HRT).

Methods: This comparative observational case series included 30 large C/D eyes (30 subjects), 17 eyes (17 patients) with early-stage normal tension glaucoma with generalized enlargement of optic disc cupping (NTG eyes), and 25 eyes from 25 normal subjects (normal eyes). Results with Humphrey 30-2, FDT N-30 threshold programs, and HRT were compared among these groups. Large C/D eyes were subdivided into FDT-normal and -abnormal eyes according to the predetermined criteria and HRT parameters were compared among them.

Results: No significant difference was seen in HRT parameters between the large C/D and NTG eyes. In the large C/D eyes, FDT mean deviation was lower than in the normal eyes and higher than in the NTG eyes, whereas FDT pattern standard deviation was smaller than in the NTG eyes ($P = 0.02$–0.03). Among HRT parameters, only cup shape measure (CSM) showed significant negative correlation with FDT mean deviation in the large C/D eyes. Between FDT-normal and -abnormal subgroups, only CSM showed significant difference ($P < 0.01$).

Conclusion: Frequency doubling technology showed abnormalities in large C/D eyes. Only CSM showed significant correlation with FDT result and difference between those with normal and abnormal FDT results. In management of large C/D eyes, FDT and CSM will be useful to detect functional and structural change.

Key Words: cup shape measure, frequency doubling technology, the Heidelberg Retina Tomograph, large C/D eyes, large cup/disc ratio, normal-tension glaucoma, scanning laser tomography, standard achromatic automated perimetry

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INTRODUCTION

Early structural abnormalities of the optic nerve head and nerve fiber changes usually precede clinically detectable visual field loss measured by standard achromatic automated perimetry (SAP).[1–7] Therefore, careful detection of structural changes in the optic nerve head is important for early diagnosis of glaucoma. In clinical practice, we sometimes encounter an eye with generalized enlargement of optic disc cupping (cup/disc ratio ≥0.8) but no other findings suggestive of glaucoma, such as a nerve fiber layer bundle defect or localized rim thinning, and with both normal intraocular pressure (IOP) and SAP results. However, there are no definitive methods to determine whether such a disc represents a variant of a normal disc or very early-stage normal tension glaucoma (NTG).

There are several psychophysical tests that might detect visual field abnormalities in patients with glaucoma years earlier than SAP, short-wavelength automated perimetry (SWAP),[8,9] high pass resolution perimetry,[10] motion perimetry,[11] or frequency doubling technology (FDT).[12–14] According to one report,[14] FDT distinguishes patients with glaucoma from normal subjects more often than SWAP or motion perimetry. Compared with SAP, the test-retest variability of FDT is lower with a shorter test time.[15]

Laser scanning tomography provides three-dimensional, highly reproducible measurements of the optic nerve head.[16,17] Several studies indicate that topographic optic disc parameters obtained using this technique can distinguish glaucomatous discs from normal discs with relatively high sensitivity and specificity.[18–20]

In the present study, we sought both to evaluate whether there were abnormalities in FDT perimetry in eyes with generalized enlargement of optic disc cupping with a vertical

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cup/disc ratio ≥0.8 but with no other ophthalmoscopic and SAP findings suggestive of glaucoma (large C/D eyes) and to determine the relationship of the abnormalities to the optic disc topography results obtained using a Heidelberg Retina Tomograph (HRT, Heidelberg Engineering GmbH, Heidelberg, Germany).

SUBJECTS AND METHODS

The study protocol was approved by the ethical review committees of the individual institutes and adhered to the tenets of the Declaration of Helsinki. Subjects were recruited consecutively from those seen in the outpatient clinics of the Departments of Ophthalmology of the University of Tokyo Graduate School of Medicine, Tajimi Municipal Hospital, Nihon University, School of Medicine, and Yoshikawa Eye Clinic from October 1998 to September 2000. We recruited subjects with an eye with generalized enlargement of optic disc cupping. After obtaining informed consent from all participants, the enrolled subjects underwent a routine ocular examination, which included visual acuity testing, slit-lamp biomicroscopy, Goldmann applanation tonometry, funduscopy with pupil dilation, and automated perimetry using a Humphrey Field Analyzer (HFA) with the central 30-2 standard full-threshold strategy (HFA30-2, Carl Zeiss Meditec, Dublin, CA). All subjects enrolled in the study were familiarized with automated perimetry by undergoing at least 2 visual field examinations with the HFA 30-2 in each eye before we obtained the data used for analysis, and all had reliable visual field measurements with fixation loss, and false-positive and false-negative rates of less than 25%. Scanning laser tomography using HRT was performed according to standard procedures. Stereoscopic optic disc photographs were obtained after mydriasis using a simultaneous stereoscopic fundus camera (Topcon TRC-SS, Topcon Inc., Tokyo, Japan) with ASA 100 36-mm film (Fuji chrome 100, Fuji Film Inc., Tokyo, Japan).

Subject Selection

Eye with a Generalized Enlargement of Optic Disc Cupping (Large C/D Eyes)

One hundred twenty eyes of 60 subjects who met the following criteria were extracted from all the subjects recruited: (1) eyes with generalized enlargement of optic disc cupping with a vertical cup/disc ratio ≥0.8 and no other apparent ophthalmoscopic signs suggestive of glaucoma, such as disc hemorrhage, nerve fiber layer bundle defects, rim notching, localized rim thinning, saucerization of the rim, or bilateral asymmetry of the cup/disc ratio ≥0.2; (2) eyes with no disc anomalies such as a tilted disc; (3) recorded IOP consistently less than 21 mm Hg bilaterally; (4) best-corrected visual acuity (BCVA) equal to or greater than 20/25; (5) no media opacity; (6) refractive errors in spherical equivalents within ± 3 diopters; (7) normal open angles bilaterally; (8) no apparent history of disc hemorrhage in either eye; (9) disc area less than 4 mm^2 measured by scanning laser tomography; and (10) no visual field loss determined by the Humphrey 30-2 program (HFA30-2) in either eye (Fig. 1). The rim border was determined by graduation of color, texture, and the course of the blood vessels based on the stereoscopic optic disc photographs, and the vertical cup/disc ratio was evaluated in 0.05 units using a ruler. The visual field was considered normal if it did not meet the modified liberal criteria proposed by Caprioli[21]; that is, (a) at least 2 adjacent test points (excluding the outermost rim) having a sensitivity with a probability of less than 5% on the pattern deviation probability plot, and/or (b) a point having a sensitivity with a probability less than 1% on the pattern deviation probability plot, and/or (c) a 5-dB difference in pattern deviation value across the nasal horizontal meridian in 2 adjacent test points.

The stereoscopic optic disc photographs of the 120 eyes then were reevaluated by 3 glaucoma specialists (MA, YY, YS) who reviewed all photographs independently in a blinded manner. If all 3 agreed that the eye had generalized enlargement of optic disc cupping with vertical cup/disc ratio ≥0.8 and no other apparent signs suggestive of glaucoma in both the studied and contralateral eyes such as disc hemorrhage, nerve fiber layer bundle defects, rim notching, localized rim thinning, saucerization of the rim, or bilateral asymmetry of the cup/disc ratio ≥0.2, that eye was enrolled in the study. When both eyes of a patient met the criteria, one randomly chosen eye was included. Thirty eyes of 30 subjects (mean age, 51.4 ± 11.1; range, 28–71) were ultimately enrolled.

Eyes with Early-Stage Normal Tension Glaucoma

We reviewed stereoscopic optic disc photographs of 282 eyes of 141 consecutive patients with NTG with mild visual field loss who had been seen in the outpatient clinic of the Department of Ophthalmology of the University of Tokyo Graduate School of Medicine. We selected eyes with generalized enlargement of optic disc cupping (generalized enlargement of the optic cup discs)[22] and an HFA 30-2 mean deviation of >−5 dB. An optic disc with generalized enlargement of the optic cup disc with a vertical C/D ratio ≥0.8 was identified based on independent readings by 3 glaucoma specialists (SM, GT, NK), and the eye was enrolled in the study only when the 3 reviewers agreed (Fig. 2). Further, the enrolled eyes with NTG had to have a BCVA of at least 20/25 with no media opacities, spherical equivalent refractive errors within ±3 diopters, and no clinically significant visual field changes during the past 2 years. Seventeen eyes of 17 patients with NTG (mean age, 59.4 ± 10.3; range 28–75) were ultimately enrolled.

An abnormal visual field in the current study for selecting NTG was defined by modifying the moderate criteria of Caprioli[21]; that is, (a) 3 or more adjacent test points (excluding the outermost rim) having a sensitivity with a probability of less than 5% on the pattern deviation probability plot, and/or (b) 2 or more adjacent points having a sensitivity with a probability less than 1% on the pattern deviation probability plot, and/or (c) a 10-dB difference in pattern deviation across the nasal horizontal meridian in 2 or more adjacent test points. When both eyes of a patient met the criteria, one randomly chosen eye was included.

A diagnosis of NTG was made in eyes with the following:

1. an untreated peak IOP no higher than 21 mm Hg during follow-up that included measurement over a 24-hour period (IOP measured every 2 hours) in both eyes

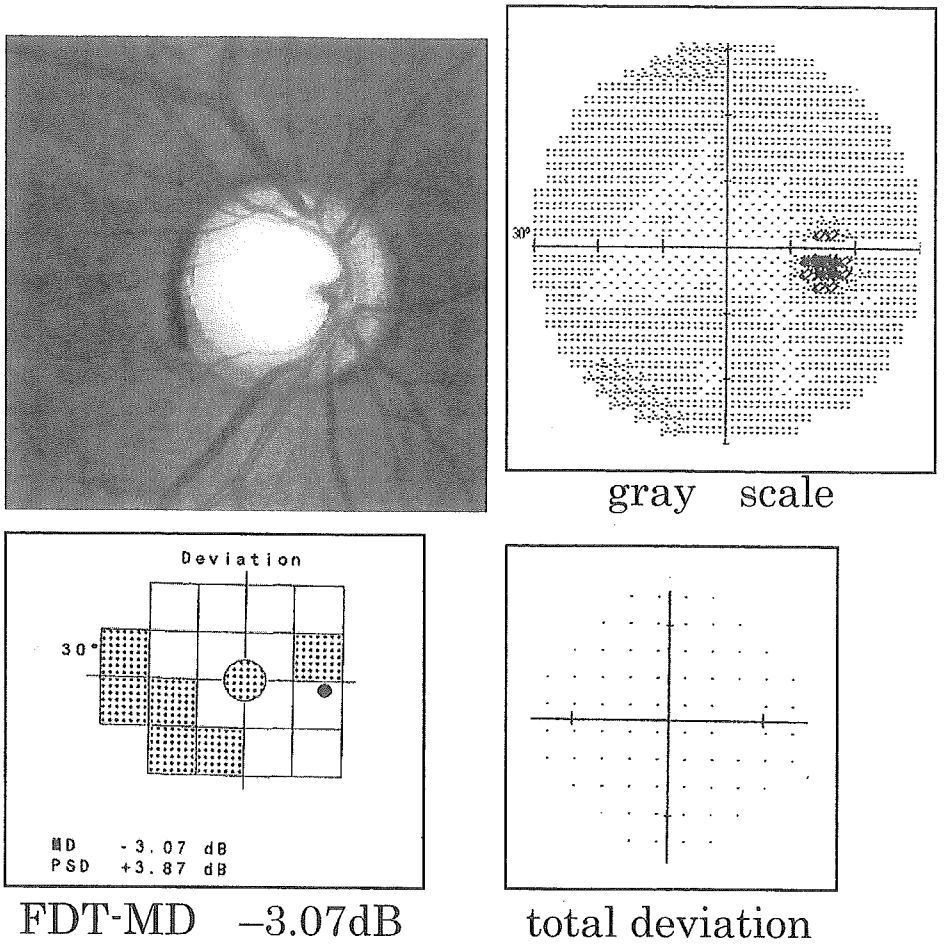

FIGURE 1. Optic disc photograph (top left), FDT printout (lower left), and HFA results (top right, gray scale; bottom right, total deviation) from a representative case of the large C/D eyes.

2. a normal open anterior chamber angles in both eyes
3. the presence of glaucomatous optic nerve head changes and corresponding visual field changes
4. no ocular, rhinologic, neurologic, or systemic disorders responsible for the optic nerve damage
5. no history of hemodynamic crisis or previous ocular surgeries or laser treatment in either eye.

Normal Eyes

The normal eyes were defined as having a BCVA of at least 20/25, refractive errors in spherical equivalents within ± 3 diopters, normal appearance of the optic disc in both eyes with a vertical cup/disc ratio ≤0.6, a normal open angle, an IOP less than 21 mm Hg in both eyes, no family history of glaucoma, no other significant ocular diseases except for a mild refractive error, no history of increased IOP or ocular trauma, and normal results with the HFA 30-2 based on the modified liberal criteria by Caprioli[21] described previously. From visual field examinations with the HFA 30-2 performed by volunteers familiar with the examination, one randomly chosen eye from 25 normal eyes (9 males and 16 females; mean age, 44.2 ± 13.5 years) were enrolled. Eyes with an apparently small optic disc were excluded.

Scanning Laser Tomography

Scanning laser tomography was performed using HRT with software version 2.01 (Heidelberg Engineering, GmBH, Heidelberg, Germany) within 3 months of the last Humphrey visual field test. The principles and description of the instrument have been reported previously.[16–20] Images with a standard deviation of less than 30 μm were used. The contour line of the optic disc margin was outlined (along the inner margin of the scleral ring) by an experienced operator (SK) while viewing photographs of the optic disc. According to software version 2.01, a reference plane was automatically set 50 μm posterior to the mean height of the disc margin contour line in a temporal segment between 350 and 356 degrees.

To analyze the configuration of the optic nerve head, we used the following HRT parameters: disc area (mm^2), cup volume (mm^3), rim volume (mm^3), mean cup depth (mm), cup shape measurement (CSM), height variation contour, and mean retinal nerve fiber layer thickness (mm).

Frequency Doubling Technology Testing

All subjects were first tested with the C-20 screening program to familiarize them with FDT using the Humphrey FDT screener (Carl Zeiss Meditec), after which they underwent the N-30 full threshold program. FDT was performed first in

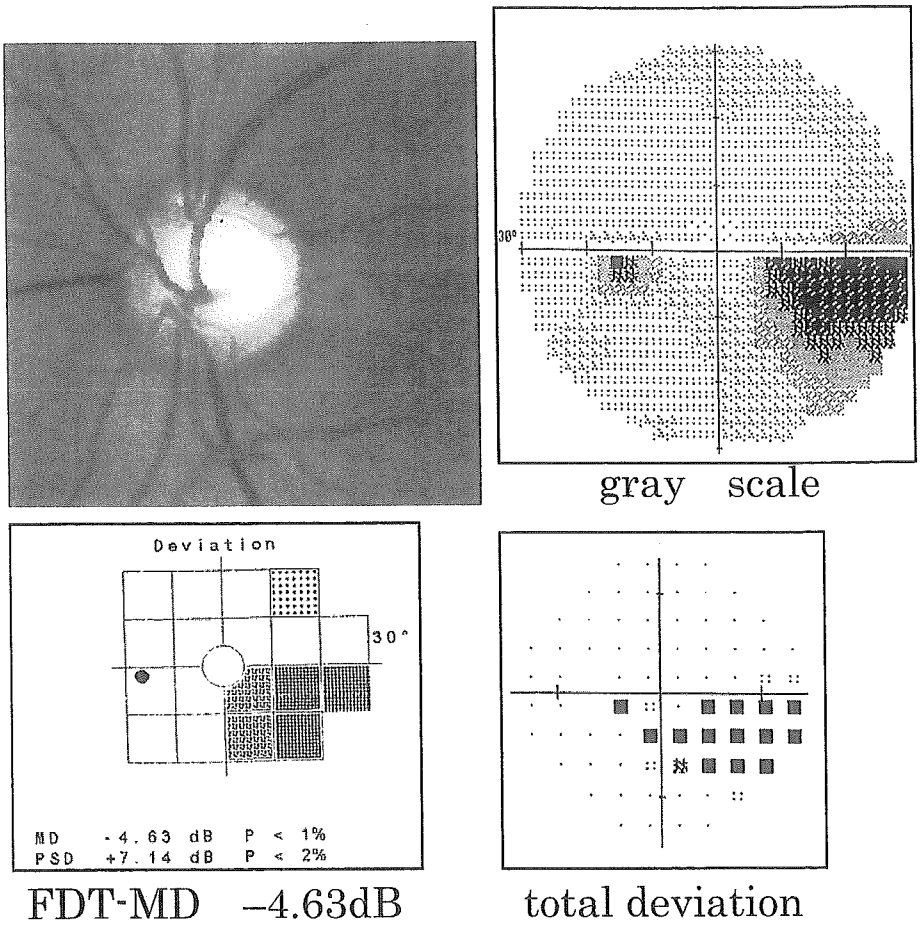

FIGURE 2. Optic disc photograph (top left), FDT printout (lower left), and HFA results (right top, gray scale; bottom right, total deviation) from a representative case with early-stage NTG.

the right eye. With the N-30 full threshold program, 19 sectors were tested, a central round sector, 16 square sectors in the periphery up to 20 degrees, and 2 more square sectors in the nasal periphery from 20 degrees up to 30 degrees. The location for each stimulus presentation was randomly selected, and the threshold value for each test location was defined by the minimal contrast of the pattern that was perceived. Patients were instructed to fixate on a small central black square on the video monitor and asked to respond when they detected a presented stimulus. There was a rest period of at least 5 minutes between the C-20 screening program and the N-30 full threshold program. The results of FDT used for analysis were obtained within 3 months of the Humphrey visual field test. To be considered reliable, a test had to have both false-positive and false-negative trials and fixation losses of less than 20%, which were calculated as in standard threshold perimetry.[23]

Wu et al[13] reported criteria for judging the N-30 results obtained from upper or lower hemifield as normal or abnormal; that is, a normal hemifield had zero or one abnormal FDT sectors at probabilities of 5%, 2%, or 1% and no abnormal sectors at a probability of 0.5%, and an abnormal hemifield had 2 or more abnormal FDT sectors at probabilities of 5%, 2%, or 1%, or one or more abnormal FDT sectors at a probability of 0.5%.

The results with the N-30 program were considered abnormal when at least one hemifield was abnormal or the result of the central round sector was abnormal (probability of <5%), and the results were considered normal when both hemifields were considered normal based on the criteria of Wu et al[13] and the results of the central round sector were normal.

Statistical Analysis

The results from large C/D eyes, early-stage NTG, and normal eyes were compared. For statistical analysis, the difference in the mean values among the 3 groups was evaluated using a two-tailed t test or a t test with Tukey's correction for multiple comparison tests (SAS software version 6.12). A difference in the observed counts between two groups was evaluated using a χ^2 test. Pearson correlation was calculated to assess the correlation between HRT parameters and the FDT mean deviation. A P value less than 0.05 was considered statistically significant.

RESULTS

Study Group Characteristics

Table 1 shows the demographics of the 31 men and 41 women included in the study. The mean age of the 30 subjects with large C/D eyes was significantly older than that of the 25 normal subjects and significantly younger than that of the 17 patients with early-stage NTG. The mean IOP was

TABLE 1. Study Population Characteristics (mean ± SD)

Variable	Large C/D Eyes (1) (n = 30)	NTG Eyes (2) (n = 17)	Normal Eyes (3) (n = 25)	1 vs 2	1 vs 3	2 vs 3
Age (years)	51.4 ± 11.1	59.4 ± 10.3	44.2 ± 13.5	*	*	*
Eye right/left	16/14	7/10	15/10			
IOP (mm Hg)	14.5 ± 2.2	16.3 ± 1.8	13.2 ± 2.3			*
Refractive error (D)	−0.91 ± 1.76	−1.08 ± 2.59	−0.46 ± 1.23			
HFA-MD (dB)	−0.87 ± 1.61	−3.49 ± 1.35	0.03 ± 1.61	*		*

HFA-MD, Humphrey field analyzer mean deviation.
*Significant difference ($P < 0.05$, t-test with Tukey's correction for multiple comparison). Large C/D eyes, eyes with generalized enlargement of optic disc cupping (vertical cup/disc ratio ≥0.8), normal intraocular pressure, normal standard achromatic automated perimetry (SAP) result, and no other ophthalmoscopic findings suggesting glaucoma. NTG eyes, eyes with early-stage normal tension glaucoma with generalized enlargement of optic disc cupping (vertical C/D ratio ≥0.8).

significantly higher in the early-stage NTG group. There was no significant inter-group difference in refraction or right/left ratio. The mean deviation of the HFA-30 program of the large C/D eyes was significantly better than that in the early-stage NTG eyes, while it was not significantly different from that in the normal eyes (Table 1).

HRT Parameters

Table 2 lists the means and standard deviations of the HRT parameters for the 3 groups of eyes. There was no significant difference in the HRT parameters between the large C/D and the early-stage NTG eyes, while the normal eyes significantly differed from the other two groups in all HRT parameters, except for the disc area. All large C/D eyes and 16 of 17 of the early-stage NTG eyes (94.1%) were classified as glaucomatous using the HRT classification program, while 1 of 25 normal eyes (4%) was classified as glaucomatous.

FDT Results

The FDT mean deviation in the large C/D eyes was significantly lower than in the normal eyes and higher than in the early-stage NTG eyes (Table 3). FDT pattern standard deviation in the large C/D eyes was not significantly different from that in the normal eyes, but it was significantly smaller than that in the early-stage NTG eyes (Table 3). Based on the criteria of Wu et al,[13] the results of FDT perimetry using N-30 program were considered abnormal in 57%, 82%, and 20% of the large C/D eyes, early-stage NTG, and normal eyes, respectively. The percentage of eyes considered abnormal by FDT was significantly higher in the large C/D eyes than in the normal eyes, but it was not significantly different between the large C/D eyes and the early-stage NTG eyes (Table 3). Among the HRT parameters examined, only the cup shape measure showed significant correlation with FDT mean deviation ($r = -0.452$, $P = 0.0140$) in the large C/D eyes, while no HRT parameters showed significant correlation with FDT mean deviation or pattern standard deviation in normal and early-stage NTG eyes.

Comparison Between Eyes With Large C/D Eyes with Normal and Abnormal FDT Results

We divided the large C/D eyes into 2 groups, FDT-normal and FDT-abnormal, using FDT mean deviation or FDT pattern standard deviation values with a probability of less than 5% as a cut-off value,[24] or the criteria of Wu et al,[13] and the HRT parameters between the two subgroups were compared. The intraocular pressure was significantly higher in the FDT-abnormal group than in the FDT-normal group when the FDT mean deviation criterion or the criteria of Wu et al[13] was applied (13.9 ± 2.5 versus 15.6 ± 1.6 mm Hg, $P = 0.045$ and 13.4 ± 2.5 versus 15.3 ± 1.6 mm Hg, $P = 0.031$, respectively).We found that only the CSM was significantly higher (ie, the cupping was steeper or more glaucomatous) in the FDT-abnormal group than in the FDT-normal group when the FDT mean deviation criterion or the criteria of Wu et al[13] was applied, while there were no significant intergroup differences in any other parameters (Tables 4–6). On the other

TABLE 2. Optic Nerve Topography in Eyes With Large C/D Eyes, NTG Eyes, and Normal Eyes (mean ± SD)

Variable	Large C/D Eyes (1) (n = 30)	NTG Eyes (2) (n = 17)	Normal Eyes (3) (n = 25)	1 vs 2	1 vs 3	2 vs 3
Disc area (mm^2)	2.88 ± 0.47	2.78 ± 0.39	2.66 ± 0.29			
Cup volume (mm^3)	0.61 ± 0.39	0.62 ± 0.42	0.19 ± 0.13		*	*
Rim volume (mm^3)	0.21 ± 0.09	0.18 ± 0.11	0.50 ± 0.12		*	*
Mean cup depth (mm)	0.38 ± 0.13	0.39 ± 0.13	0.26 ± 0.08		*	*
CSM	−0.04 ± 0.06	−0.03 ± 0.06	−0.20 ± 0.07		*	*
Height variation contour (mm)	0.35 ± 0.09	0.35 ± 0.11	0.41 ± 0.07		*	*
Mean RNFL thickness (mm)	0.17 ± 0.06	0.17 ± 0.07	0.27 ± 0.05		*	*

CSM, cup shape measure; RNFL, retinal nerve fiber layer.
*Significant difference ($P < 0.05$, t-test with Tukey's correction for multiple comparison) for explanation of large C/D eyes and NTG eyes. See footnotes for Table 1.

TABLE 3. FDT in Eyes With Large C/D Eyes, NTG Eyes, And Normal Eyes (mean ± SD)

Variable	Large C/D Eyes (1) (n = 30)	NTG Eyes (2) (n = 17)	Normal Eyes (3) (n = 25)	1 vs 2	1 vs 3	2 vs 3
FDT- MD (dB)	−2.41 ± 2.30	−3.96 ± 2.23	−0.42 ± 2.06	*	*	*
FDT-PSD (dB)	4.46 ± 2.06	6.38 ± 2.57	3.70 ± 0.55	*		*
Criterion of Wu et al[13] (abnormal/normal)	17/13	14/3	5/20		†	†

FDT, frequency doubling technique; FDT-MD, frequency doubling technique mean deviation; FDT-PSD, frequency doubling technique pattern standard deviation.
*Significant difference ($P < 0.05$, t-test with Tukey's correction for multiple comparison); †Significant difference ($P < 0.05$, χ^2 test).

hand, when the FDT pattern standard deviation criterion was used, there were no significant intergroup differences in HRT parameters, probably because only 5 eyes were judged to be FDT pattern standard deviation abnormal.

DISCUSSION

A physiologic large cup/disc ratio is a diagnostic term applied to eyes with generalized enlargement of optic disc cupping and normal IOP but no other evidences suggestive of glaucoma.[25] Clinically, however, it sometimes may be difficult to differentiate pre-perimetric stage NTG eyes with generalized enlargement of optic disc cupping from normal variant eyes with a large cup/disc ratio. The prevalence of NTG is thought to be much higher in Japan than in other countries.[26] It is possible that some eyes with a large cup/disc ratio may represent pre-perimetric-stage NTG eyes. The current study was designed to find clues to identify eyes with large C/D that appears otherwise healthy but may be actually in pre-perimetric stage of NTG. For this purpose, we included only eyes with generalized enlargement of optic disc cupping with a vertical cup/disc ratio ≥0.8 (large C/D eyes). For NTG eyes, we thought that it is more appropriate to use NTG eyes closer to pre-perimetric stage; that is, in the earlier stage of the disease, and with similar appearance of optic nerve head (generalized enlargement of optic disc cup with a vertical C/D ratio ≥0.8).

In the current study, the average age happened to be somewhat different among the 3 groups compared. We think, however, this difference has little influence on the validity of comparison performed for the following reasons:

1. The most important intergroup comparison in the current study was that between large C/D eyes with normal FDT result and those with abnormal FDT result. As long as this comparison was concerned, no intergroup age difference was found (Tables 4–6).
2. Regarding HFA and FDT results, we used age-corrected parameters.
3. In Japanese eyes, age-related differences in HRT parameters presently used should be little between the age of 44 and 59 years.[27]

Our findings suggested that FDT N-30 results are useful for differentiating the large C/D eyes as in the current study from both normal eyes and those with early-stage NTG with generalized enlargement of optic disc cupping, and that some of large C/D eyes as in the current study have functional abnormalities in the M-cell pathway that is more sensitively detected by FDT.[28,29] In both the normal and early-stage NTG eyes, no HRT parameters showed correlation with FDT mean deviation, while only CSM showed significant correlation with FDT mean deviation in the large C/D eyes. We carried out this correlation analysis for exploratory purposes. Since we calculated correlation coefficients for 6 HRT parameters excluding disc area, conservative P value may be $0.084 = 0.014 \times 6$ according to the Bonferroni correction. Being compatible with this result, when the large C/D eyes were divided into the FDT-normal and FDT-abnormal subgroups based on the FDT mean deviation or criteria of Wu et al,[13] the CSM was significantly higher (ie, cupping was steeper) and

TABLE 4. HDT Parameters in FDT-Abnormal Subgroup and FDT-Normal Subgroup in Large C/D Eyes by FDT Mean Deviation Criterion (mean ± SD)

Variable	FDT-Abnormal Group (n = 10)	FDT-Normal Group (n = 20)	*P* Value
Disc area (mm^2)	2.91 ± 0.41	2.85 ± 0.45	0.747
Cup volume (mm^3)	0.71 ± 0.39	0.58 ± 0.42	0.422
Rim volume (mm^3)	0.20 ± 0.09	0.23 ± 0.09	0.391
Mean cup depth (mm)	0.40 ± 0.12	0.37 ± 0.13	0.068
CSM	0.00 ± 0.05	−0.07 ± 0.05	0.002
Height variation contour (mm)	0.35 ± 0.08	0.36 ± 0.09	0.801
Mean RNFL thickness (mm)	0.17 ± 0.07	0.17 ± 0.06	0.999
Age (years)	55.6 ± 10.1	48.8 ± 5.2	0.067

CSM, cup shape measure; RNFL, retinal nerve fiber layer; HRT, Heiderberg Retina Tomograph; FDT, frequency doubling technique.

TABLE 5. HRT Parameters in FDT-Abnormal Subgroup and FDT-Normal Subgroup in Large C/D Eyes by Criterion of Wu et al[13] (mean ± SD)

Variable	FDT-Abnormal Group (n = 17)	FDT-Normal Group (n = 13)	*P* value
Disc area (mm^2)	2.96 ± 0.40	2.77 ± 0.47	0.252
Cup volume (mm^3)	0.77 ± 0.48	0.44 ± 0.19	0.028
Rim volume (mm^3)	0.21 ± 0.10	0.23 ± 0.08	0.566
Mean cup depth (mm)	0.41 ± 0.16	0.32 ± 0.07	0.271
CSM	−0.01 ± 0.05	−0.08 ± 0.05	<0.001
Height variation contour (mm)	0.36 ± 0.08	0.36 ± 0.11	0.801
Mean RNFL thickness (mm)	0.17 ± 0.07	0.18 ± 0.06	0.818
Age (years)	54.0 ± 10.7	51.6 ± 10.2	0.533

HRT, Heiderberg Retina Tomograph; FDT, frequency doubling technique; CSM, cup shape measure; RNFL, retinal nerve fiber layer.

the IOP also higher in the FDT-abnormal group. The possibility that higher IOP affected the FDT N-30 test results may not be excluded, but rather seems unlikely. The IOPs in both FDT normal and abnormal eyes were close to the normal average IOP (about 13.5 and 15.5 mm Hg, respectively).[30] CSM is a description of depth; cups with gradually sloping borders tend to have negative values and cups with steep sloping walls tend to have positive values. CSM is an important HRT parameter that indicates the degree of glaucomatous disc damage[18] (more positive CSM value). Furthermore, several investigators have suggested that CSM is the single best HRT parameter for distinguishing between normal eyes and those with early-stage glaucoma.[31–34] Taken together, the present findings suggest that FDT mean deviation, CSM, or both might be clinically useful for grading the abnormalities in eyes with generalized enlargement of optic disc cupping with vertical cup/disc ratio ≥0.8.

It is tempting to speculate that large C/D eyes as in the current study with normal SAP and IOP values but with abnormal FDT results are more likely to develop NTG in the future, but a longitudinal study is needed to evaluate this possibility.

TABLE 6. HRT Parameters in FDT-Abnormal Subgroup and FDT-Normal Subgroup in Large C/D Eyes by FDT-PSD Criterion (mean ± SD)

Variable	FDT-Abnormal Group (n = 5)	FDT-Normal Group (n = 25)	*P* Value
Disc area (mm^2)	2.88 ± 0.47	2.88 ± 0.43	0.996
Cup volume (mm^3)	0.82 ± 0.56	0.59 ± 0.38	0.240
Rim volume (mm^3)	0.23 ± 0.12	0.21 ± 0.08	0.846
Mean cup depth (mm)	0.48 ± 0.15	0.36 ± 0.11	0.080
Cup shape measure	−0.04 ± 0.04	−0.04 ± 0.09	0.805
Height variation contour (mm)	0.38 ± 0.08	0.36 ± 0.09	0.559
Mean RNFL thickness (mm)	0.18 ± 0.07	0.18 ± 0.07	0.704
Age (years)	51.4 ± 9.8	58.8 ± 11.6	0.144

HRT, Heiderberg Retina Tomograph; FDT, frequency doubling technique; RNFL, retinal nerve fiber layer.

The average disc area in the present study was 2.88 ± 0.47 mm^2 in the large C/D eyes, which is somewhat larger than the average disc in Japanese patients (2.22 mm^2).[27] Heijl and Molder[35] studied the effect of optic disc diameter on the diagnostic power of subjective disc evaluation in glaucoma. The sensitivity for recognizing glaucoma was 58% in the small disc group, 72% in the middle group, and 85% in the large disc group. Thus, the larger discs were more likely to be judged as glaucomatous. In other words, patients with large discs have a greater chance of being diagnosed with glaucoma than patients with medium-sized or small discs. It is possible that there was a similar bias in the eyes in which we detected a large cup/disc ratio. There was no significant difference in disc size, however, between the current large C/D eyes and the early-stage NTG or normal eyes in this study. The differences in the diagnosis between these three groups should not depend on disc size.

Mansberger et al[8] investigated the difference in HRT parameters in large C/D eyes (vertical C/D ≥0.8) with SAP abnormalities and those with only short-wavelength automated perimetry (SWAP) abnormalities and found that the optic nerve head appearance in the latter group was less glaucomatous. In the current study, the parallel of the former group in the study of Mansberger et al[8] would be the early-stage NTG group and that of the latter group of their study the large C/D eyes with FDT abnormalities. In the current study, however, no HRT parameters showed significant difference between the above two groups. This apparent discrepancy may be at least partly attributed to difference in the IOP of subject eyes between the two studies (high IOP subjects in the study of Mansberger et al[8] and normal IOP subjects in the current study) or difference between FDT being more sensitive to change in M-cell pathway and SWAP being more sensitive to change in short-wavelength sensitive pathway.[36] Whether SWAP result is also useful for differentiating the large C/D eyes as in the current study from normal and early-stage NTG eyes with generalized enlargement of optic disc cupping waits future studies.

In summary, there was no significant difference in the HRT parameters between the current large C/D eyes and the eyes with early-stage NTG with generalized enlargement of optic disc cupping. The results using the FDT N-30 program in the large C/D eyes were significantly different from both the early-stage NTG and normal eyes. When the HRT parameters were compared between the two subgroups of the large C/D eyes, that is, those with FDT-normal results and those with FDT-abnormal results, only the CSM was significantly higher (ie, cupping was steeper) in eyes with FDT-abnormal results. Further, among HRT parameters, only the CSM showed significant negative correlation with FDT mean deviation in this group. Therefore, a part of eyes with a large cup/disc ratio but without any other signs suggestive of glaucoma as in this study may already have functional and/or structural change that can be detected more sensitively by FDT than SAP, and by CSM. Longitudinal follow-up of these eyes will help determine the predictive value of FDT for future development of NTG.

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Intraocular Metabolites of Isopropyl Unoprostone

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ABSTRACT *Purpose*: It is still unknown which metabolite of isopropyl unoprostone is responsible for reducing intraocular pressure. This study was carried out to measure intraocular metabolites of isopropyl unoprostone in the aqueous humor of primate and human eyes. *Methods*: Nine monkeys were randomly divided into three groups, all of which received isopropyl unoprostone. In group I, the drug was scheduled to be instilled at 0 hr, in group II at 1 hr, and in group III at 2 hr, prior to aqueous humor aspiration in order to determine metabolite concentration. Furthermore, 27 patients scheduled for cataract surgery and intraocular lens implantation were divided into five groups that received isopropyl unoprostone. In group A, the drug was scheduled to be instilled at 0 hr, in group B at 1 hr, in group C at 2 hr, in group D at 3 hr, and in group E at 4 hr, prior to surgery. At the beginning of the operation, the aqueous humor was aspirated. Metabolites of isopropyl unoprostone in the aqueous humor were determined by high-performance liquid chromatography. *Results*: M1 (3-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-(3-oxodecyl)cyclopentyl]propionic acid) (unoprostone free acid) and M2 ((*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-(3-oxodecyl)cyclopentyl]hept-5-enoic acid), an intraocular oxidized metabolite of isopropyl unoprostone, were measured. M1:M2 in monkeys was respectively 0:0 ng/ml in group I, 150.2 ± 45.1:9.5 ± 1.7 ($p < 0.05$) in group II, and 74.6 ± 31.4:19.2 ± 5.3 ($p < 0.01$) in group III. M1:M2 in humans was respectively 0:0 ng/ml in group A, 50.6 ± 22.3:3.2 ± 1.3 ($p < 0.05$) in group B, 125.0 ± 23.1:12.2 ± 3.4 ($p < 0.001$) in group C, 144.9 ± 33.8:24.5 ± 6.2 ($p < 0.01$) in group D, and 56.7±21.5:18.7±5.3 ($p < 0.05$) in group E. *Conclusions*: A free acid of isopropyl unoprostone is the major intraocular metabolite of isopropyl unoprostone that is expected to act on target tissues in the eyes of both monkeys and humans.

KEYWORDS aqueous humor; human eye; intraocular metabolism; isopropyl unoprostone; monkey eye

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INTRODUCTION

Prostaglandin (PG) F2 α-related compounds isopropyl unoprostone, latanoprost, travoprost, and bimatoprost are now widely used as antiglaucoma ophthalmic solutions because of their efficacy in reducing intraocular pressure (IOP).[1–7] Isopropyl unoprostone was developed in Japan and became

commercially available in 1994 as the first approved PG-related antiglaucoma ophthalmic solution.[1,2] This solution is, among PG F2 α–related compounds, the weakest at reducing IOP.[3] However, isopropyl unoprostone has fewer side effects with regard to hyperpigmentation and eyelash growth than other PG F2 α–related compounds.[4]

Isopropyl unoprostone is classified as a docosanoid with low affinity to all prostanoid receptors,[8] and the mechanism by which isopropyl unoprostone reduces IOP remains unclear. Sharif *et al.* suggested that isopropyl unoprostone and its free acid act as a weak FP agonist, whereas latanoprost or travoprost act as a potent FP agonist.[8,9] Thieme *et al.* reported that free acid of isopropyl unoprostone contracts trabecular meshwork cells through the maxi-K channel.[10] Kashiwagi *et al.* measured intraocular metabolites of isopropyl unoprostone in the aqueous humor of pigmented Dutch rabbits by high-performance liquid chromatography (HPLC) and found that M2 ((*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-(3-oxodecyl)cyclopentyl]hept-5-enoic acid), an oxidized metabolite of isopropyl unoprostone, predominated in terms of concentration, production of PGE, and stimulation of melanocytes compared to M1 (3-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-(3-oxodecyl)cyclopentyl]propionic acid), a free acid of isopropyl unoprostone (Fig. 1).[11–13] Based on these results, Kashiwagi *et al.* suggested that M2 was the main pharmacological source of isopropyl unoprostone in the human eyeball. However, there may be species differences in the metabolism of PG-related compounds. Therefore, intraocular metabolites of isopropyl unoprostone in the human and primate eye remains unclear. This study was performed to measure the levels of the metabolites of isopropyl unoprostone in the aqueous humor of the primate and human eye.

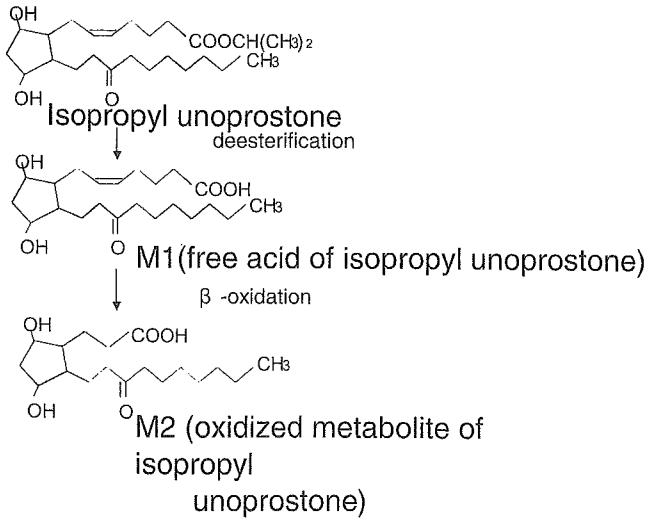

FIGURE 1 Metabolism of isopropyl unoprostone. Metabolites in the isopropyl unoprostone cascade are abbreviated M1 and M2.

MATERIALS AND METHODS

Cynomolgus Monkeys

The study was designed according to the time-course of the effects on IOP after isopropyl unoprostone instillation in cynomolgus monkeys,[14] and samples were collected up to 2 hr after eye drops were administered. A single instillation of 0.035 ml ophthalmic solution of isopropyl unoprostone containing 0.042 mg isopropyl unoprostone was administered to one randomly chosen eye of a cynomolgus monkey.

Nine cynomolgus monkeys (males 3–5 kg, 3–7 years old, Shinnihon Kagaku Co. Ltd., Tokyo, Japan) were randomly divided into three groups that received isopropyl unoprostone. In group I, the drug was scheduled to be instilled at 0 hr, in group II at 1 hr, and in group III at 2 hr prior to aspiration of 0.1 ml aqueous humor in order to determine isopropyl unoprostone metabolites. All samples were supplemented with 100% acetonitrile at double the sample volume and then frozen at −20°C until analysis. All procedures were carried out in compliance with the ARVO Statement for the Care and Use of Animals in Ophthalmology and Vision Research.

Humans

Twenty-seven patients (male/female: 6/21, mean age ± SD: 78.1 ± 8.1 years) scheduled for cataract surgery and intraocular lens implantation were enrolled in the study between February and November 2003 at the Tokyo Metropolitan Geriatric Hospital (Tokyo, Japan). Written informed consent was obtained from all participants, and the Ethics Committee of Tokyo Metropolitan Geriatric Hospital approved the study protocol; the study was performed according to the principles of the Helsinki Declaration. A single instillation of 0.035 ml ophthalmic solution of isopropyl unoprostone containing 0.042 mg isopropyl unoprostone was administered to a patient's eye. The patients were randomly divided into five groups that received isopropyl unoprostone. In group A, the drug was scheduled to be instilled at 0 hr, in group B at 1 hr, in group C at 2 hr, in group D at 3 hr and in group E at 4 hr prior to surgery. The pupil was routinely dilated with one drop

of tropicamide and phenylephrine hydrochloride, both given 4 times at intervals of 30 min before the surgery in all 5 groups. At the beginning of the operation, 0.1 ml of aqueous humor was aspirated for the determination of drug concentration. All samples were supplemented with 100% acetonitrile at double the sample volume and then frozen at −20°C. until analysis.

High-Performance Liquid Chromatography and Mass Spectrometry Operating Conditions

In Kashiwagi's results from rabbits, intraocular metabolites of isopropyl unoprostone other than M1 or M2 were barely detectable between 5 min and 12 hr after topical isopropyl unoprostone administration.[11] Therefore, this study measured M1 and M2 as follows. Aqueous humor samples were assayed using an HPLC-tandem mass spectrometry (LC-MS/MS) method.[15,16] The lower limit of quantitation was 5.0 ng/ml for isopropyl unoprostone and 1.0 ng/ml for M1 and M2. HPLC was performed using an 1100 series liquid chromatograph system equipped with a G1322A degaser, G1312A pump, and G1313A autosampler (Agilent Technologies Italia, Milan, Italy). The analytical column was a Develosil ODS-UG-5 cartridge with a 5-μm particle size and 50 mm × 2 mm internal diameter (Nomura Chemical Co., Ltd., Aichi, Japan); it eluted with a mixture of (A) 0.1% acetic acid in 20% acetonitrile and (B) 0.1% acetic acid in acetonitrile as the mobile phase. Separation was obtained using 0–80% linear gradients of B in A for 2 min, followed by isocratic elution with 80% B for 3 min at a flow rate of 0.25 ml/min. The LC eluate was introduced directly into an API365 MS (Sciex, Thornhill, Ontario, Canada) equipped with a Turbo Ionspray (Concord, Canada). The negative turbo-ionspray voltage (V) was −4800 mV. The dwell time was 100 ms. Total run time was 8 min, and the obtained multiple reaction monitoring chromatograms were used for quantitation. A weighted linear least-squares regression was used to generate calibration curves from standards and calculate the concentrations of quality control (QC) samples. The peak area was measured using MacQuan software (version 1.6).

Statistical Analyses

Statistical analyses were conducted using a Student's *t* test. Mean and standard error have been indicated. A p value of less than 0.05 was considered statistically significant.

RESULTS

Isopropyl unoprostone was not detected in the aqueous humor of cynomolgus monkeys. M1:M2 in the monkey was respectively 0:0 ng/ml in group I (n = 3), 150.2 ± 45.1:9.5 ± 1.7 ($p < 0.05$) in group II (n = 3), and 74.6 ± 31.4:19.2 ± 5.3 ($p < 0.01$) in group III (n = 3). The M1 concentration was much higher than that of M2 at every measuring time (Fig. 2) and M1 was found to be the main intraocular metabolite of isopropyl unoprostone in cynomolgus monkeys.

Isopropyl unoprostone was not detected in the aqueous humor of humans. M1:M2 in human was respectively 0:0 ng/ml in group A (n = 5), 50.6 ± 22.3:3.2 ± 1.3 ($p < 0.05$) in group B (n = 5), 125.0 ± 23.1:12.2 ± 3.4 ($p < 0.001$) in group C (n = 6), 144.9 ± 33.8:24.5 ± 6.2 ($p < 0.01$) in group D (n = 6), and 56.7 ± 21.5:18.7 ± 5.3 ($p < 0.05$) in group E (n = 5). M1 concentration was much higher than that of M2 at all measuring times, and M1 was found to be the main intraocular metabolite of isopropyl unoprostone in humans (Fig. 3).

DISCUSSION

The current work indicated that after drops of isopropyl unoprostone are administered, M1, a free acid of isopropyl unoprostone, is the primary intraocular metabolite in monkeys and humans. Which of the metabolized compounds to be measured was determined according to the previous study reporting types

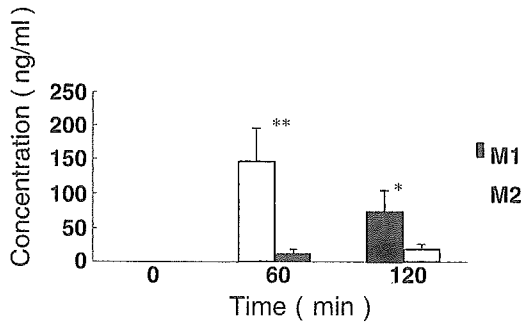

FIGURE 2 Time-course of aqueous humor concentrations of M1 and M2 in cynomolgus monkeys. M1 is (3-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-(3-oxodecyl)cyclopentyl]propionic acid), that is, a free acid of isopropyl unoprostone. M2 is ((*Z*)-7-[(1*R*,2*R*,3*R*,5*S*)-3,5-dihydroxy-2-(3-oxodecyl)cyclopentyl]hept-5-enoic acid), that is, an intraocular oxidized metabolite of isopropyl unoprostone. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. M2, n = 3, mean ± SE.

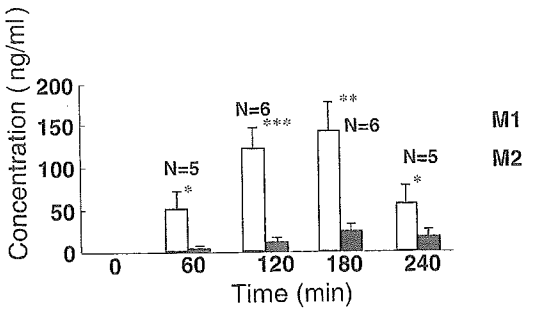

FIGURE 3 Time course of aqueous humor concentrations of M1 and M2 in humans. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs. M2, n = 5 or 6, mean ± SE.

of isopropyl unoprostone.[11] Timepoints of measurements after instillation were determined according to the reported time-course of the IOP after isopropyl unoprostone instillation in cynomolgus monkeys and humans.[1,14,17]

In both monkeys and humans, M1 was the main intraocular metabolite, but the peak time after instillation differed. Although we do not have good explanation for the difference between monkeys and humans in the time course of aqueous M1 and M2 levels after isopropyl unoprostone instillation, there may be several possible reasons. The rate of aqueous humor production in cynomolgus monkeys is somewhat lower than that in humans,[17] while the volume of the anterior chamber is thought to be much smaller than that in humans.[18] Therefore, the turnover rate of the aqueous and isopropyl unoprostone-derived materials in the aqueous is expected to be higher in cynomolgus monkeys than that in humans, resulting in an earlier peak in these materials in this species. Another possibility that cannot be excluded is that repeated instillation of mydriatics before cataract surgery affected the intracameral isopropyl unoprostone delivery in humans (cataract patients). Additionally, cynomolgus monkeys have thinner corneas than humans,[19] which might also have affected the peak time of instilled substances in the anterior chamber. Kashiwagi's study showed that M2 was dominant in the aqueous humor of rabbits.[11] Differences with Kashiwagi's results with rabbits are thought to be at least partly attributed to a species difference in the metabolism of PG-related compounds. While the current authors actually performed the same experiment using rabbits, M1 predominated within an hour of drop administration; by 2 hr, M2 was predominant, which was roughly compatible with the results of Kashiwagi *et al.*[11] Time changes in IOP after topical isopropyl unoprostone was administered were similar to those M1 levels in both primate and human aqueous, which may support that the free acid of isopropyl unoprostone, M1, is mainly responsible for ocular hypotensive effects of isopropyl unoprostone.[14] Based on the current results, isopropyl unoprostone's action on intraocular tissues such as human or primate trabecular meshwork cells, iris melanocytes, and ciliary muscle examined using M1 rather than unchanged isopropyl unoprostone or M2, an oxidized metabolite of isopropyl unoprostone, should have clinical relevance. Using the iris and ciliary bodies of rabbits and cows, Kashiwagi *et al.* suggested[12] the possibility that M2 induces a drop in IOP through PGE production. This possibility is rather unlikely at least in primates and in humans. Whether M1 lowers IOP in humans through action on maxi-k channels of trabecular meshwork (TM) cells[10] or acts as an FP agonist[8,9] awaits future studies. In pigs, isopropyl unoprostone is metabolized to M1 only by the corneal epithelial cells, while in the iris and ciliary bodies, isopropyl unoprostone underwent little metabolism.[20] If metabolism of isopropyl unoprostone in pigs is similar to that in humans and monkeys, the unchanged isopropyl unoprostone should be used rather than M1 or M2 to examine its effects on extraocular tissue (i.e., ciliary arteries, epidermal cells, and fibroblasts).[21,22] It is of interest that Yoshitomi *et al.* reported that unchanged isopropyl unoprostone, but not its metabolites M1 and M2, relaxed precontracted rabbit ciliary arteries.[21] Tamaki *et al.* reported that blood flow of the optic nerve head was increased after isopropyl unoprostone instillation in humans only in the treated site.[23] Taken together with the results reported by Yoshitomi *et al.*[21] and Babiole *et al.*[20], the result reported by Tamaki *et al.*[23] may suggest that isopropyl unoprostone relaxed short ciliary arteries also in humans.

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