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Long-term Effect of Topically Applied Isopropyl Unoprostone on Microcirculation in the Human Ocular Fundus

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Purpose: To investigate the long-term effect of 0.12% isopropyl unoprostone (Rescula®) on microcirculation in the human ocular fundus.

Methods: A laser speckle tissue circulation analyzer was used to measure normalized blur (NB), a quantitative index of blood flow velocity, in the optic nerve head (ONH) and choroid-retina before and 4.5 hours after the instillation of a placebo into both eyes of 11 healthy volunteers. The intraocular pressure (IOP), blood pressure, and pulse rate were also recorded in this control experiment. Thereafter, a drop of unoprostone or a placebo was instilled into each eye in a double-blind manner twice a day for 21 days to form treated and untreated groups.

Results: After 21 days, the NB values in the ONH and choroid-retina had increased significantly and the IOP had decreased significantly in the unoprostone-treated eyes. Ocular perfusion pressure showed no significant change.

Conclusions: These results suggest that long-term application of unoprostone can increase microcirculatory blood flow in the human ocular fundus, probably due to a reduction in vascular resistance. **Jpn J Ophthalmol 2002;46:31–35** © 2002 Japanese Ophthalmological Society

Key Words: Human eye, isopropyl unoprostone, laser speckle method, long-term topical application, microcirculation in ocular fundus.

Introduction

Circulatory disorders in the optic nerve head (ONH) are suspected to be associated with the pathogenesis and development of open-angle glaucoma and normal tension glaucoma.[1–4] Thus, it is important to determine the effect of glaucoma drugs on the microcirculation in the ocular fundus.

Isopropyl unoprostone (Rescula®, unoprostone) is a prostaglandin (PG)-related compound developed in Japan that is available for treating glaucoma and ocular hypertension. In addition to its intraocular pressure (IOP)-lowering effect, unoprostone is reported to have an effect on ocular blood flow. Using either a hydrogen gas clearance flowmeter or a thermal diffusion flowmeter, there have been reports that topically applied unoprostone increases blood flow in the ONH and choroid of rabbits.[5–9] There are also reports on blood flow in human eyes using noninvasive methods such as color Doppler imaging, laser speckle method, and ocular blood flow tonography.[10–13] Some of these studies showed that topical application of unoprostone increased while other studies showed that it did not change the ocular blood flow. However, most of these were single-dose studies.

In this study, we examined the long-term effect of unoprostone on the microcirculation in the human ONH and choroid-retina, using a laser speckle tissue circulation analyzer.

Materials and Methods

The subjects were 11 healthy volunteers, ranging in age from 34 to 59 years (mean = 43.9 years), with no

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systemic or ocular disease except mild myopia. The study was approved by the Ethics Committee of Osaka Medical College. Before admission into the study, written informed consent was obtained from each subject.

A laser speckle tissue circulation analyzer was used to evaluate microcirculation in the ocular fundus. This instrument was recently developed in Japan to analyze the capillary circulation in the retina, choroid, and optic nerve head (ONH).[14] In this method, the normalized blur (NB), blurring of a speckle pattern formed by scattered laser light, is used as a quantitative index of tissue blood flow as well as blood flow velocity.[14,15] NB was measured every 0.125 seconds and averaged over five pulses. Measurements were made on the temporal side of the ONH in an area free of surface blood vessels (NB_{ONH}), and in the middle of the choroid-retina between the ONH and macula (NB_{CHO}) where no surface vessels were visible on the monitor. Isono et al[16] reported that the square blur rate, almost equivalent to a square of NB and more linearly correlated with high speed blood velocity, is derived 25% from the retinal and 75% from the choroidal circulation in eyes with circulatory disorders. Therefore, we used the expression "choroid-retina" as the NB representing the blood flow of both retina and choroid.

On the first day (day 0, control experiment), after mydriasis of both eyes with one drop of 0.4% tropicamide at 11:00 AM, the NB, IOP, brachial arterial pressure (BP), and pulse rate (PR) were determined. Slit-lamp examinations were carried out at 12:00 PM. Then 30 μL of the vehicle of unoprostone was instilled in both eyes at 12:30 PM, and the same measurements were carried out at 5:00 pm, 4.5 hours after the instillation. The IOP was measured with the Goldmann applanation tonometer, and the BP and PR were measured with an automated sphygmomanometer (HEM 705 CP, Omron, Tokyo). The time of measurement was based on the single-dose study by Kojima et al,[11] which concluded that the NB in the ONH and choroid-retina showed a maximum increase at 4.5 hours after the instillation of unoprostone.

Thereafter, one drop of unoprostone or vehicle was instilled into each eye in a double-masked manner twice a day for 21 days (treated and untreated groups). On the 7th and 14th days (days 7 and 14), IOP measurements and slit-lamp examinations of both eyes were performed at 12:00 PM. On the final day (day 21), the NB, IOP, BP, and PR were measured as on the first day (unoprostone experiment). On the first and last experimental days, the participants were kept from smoking, eating, drinking, and exercise from 11:00 AM to 5:00 PM.

Mean BP (BP_m) was calculated by the following equation:

$$BP_m = BP_d + (1/3)(BP_s - BP_d)$$

in which BP_d and BP_s represent the diastolic and systolic BP, respectively. Ocular perfusion pressure (OPP) was calculated as:

$$OPP = (2/3)BP_m - IOP$$

Data are expressed as the means ± SD, and analyzed by paired *t*-test between the control and unoprostone-treated eyes. *P* values less than .05 were considered significant.

Results

Slit-lamp examinations revealed no side effects induced by unoprostone treatment on any experimental days.

On day 21, 4.5 hours after the last instillation of unoprostone, NB_{ONH} in the treated eyes had increased significantly compared with the value at the same time on day 0 (Table 1). Untreated eyes did not show any significant changes (Table 1). On day 21, NB_{CHO} in the treated eyes was also significantly higher before and 4.5 hours after instillation compared with the values on day 0 (Table 1). NB_{CHO} in the untreated eyes had not changed significantly (Table 1).

The IOP on day 7 and 14 did not change significantly in either eye compared with values of day 1

Table 1. Changes in NB_{ONH}, NB_{CHO}, and IOP*

	Before	4.5 Hours
Control experiment (day 0)		
NB_{ONH} (U)	8.2 ± 1.6	8.0 ± 1.3
NB_{ONH} (P)	8.4 ± 1.4	8.1 ± 1.3
NB_{CHO} (U)	9.0 ± 1.5	8.4 ± 1.2†
NB_{CHO} (P)	9.3 ± 1.9	9.1 ± 1.7
IOP_u (mm Hg)	15.6 ± 2.0	14.0 ± 2.2†
IOP_p (mm Hg)	16.1 ± 2.2	14.2 ± 2.1
Unoprostone experiment (day 21)		
NB_{ONH} (U)	8.4 ± 1.4	8.5 ± 1.4
NB_{ONH} (P)	8.3 ± 1.4	8.5 ± 1.3
NB_{CHO} (U)	9.5 ± 1.8‡	9.3 ± 1.5§
NB_{CHO} (P)	9.5 ± 1.6	9.4 ± 1.5
IOP_u (mm Hg)	13.3 ± 2.5§	12.1 ± 3.2§
IOP_p (mm Hg)	14.8 ± 2.6	13.6 ± 2.9

*NB_{ONH}: normalized blur in optic nerve head, NB_{CHO}: normalized blur in middle of choroid-retina, IOP; intraocular pressure. Unoprostone (U) and placebo (P) indicate NB values or intraocular pressure in treated and untreated eyes. Data are expressed as mean ± SD (N = 11).

†$P < .05$ (comparison with initial value, paired *t*-test).

‡$P < .05$.

§$P < .01$ (comparison with control experiment, paired *t*-test).

(data not shown). However on day 21, IOP before and 4.5 hours after instillation was significantly lower than on day 0 in treated eyes (Table 1). Untreated eyes did not show significant changes (Table 1).

BP_m, PR, and OPP did not show significant changes in either eye throughout the experimental period (Table 3).

Discussion

There have been reports on the effect of unoprostone on ocular circulation. Sugiyama et al[5] and Ogo[7,8] reported that topically applied unoprostone increased the choroidal blood flow in rabbit eyes. Furthermore, in rabbit experiments, Sugiyama et al[6,9] showed that topical application of unoprostone inhibited the decrease of ONH blood flow in circulatory disorder model eyes, which indicated a favorable effect of maintaining the ocular blood flow.

The effect of unoprostone on ocular circulation in human eyes has been studied by noninvasive methods, such as color Doppler ultrasound imaging, laser speckle method, and ocular blood flow tonography. Using color Doppler ultrasound imaging, Nishi et al[10] reported that the blood flow velocity of the central retinal artery and the short posterior ciliary arteries increased significantly after a single dose of unoprostone. Kojima et al[11] studied the effect of unoprostone on ONH and choroid-retinal circulation by the laser speckle method, and reported that NB in the ONH did not change but NB in choroid-retina increased significantly. Using ocular blood flow tonography in normal human eyes, Kitaya et al[12] reported that a single dose of unoprostone did not change the pulsatile ocular blood flow significantly. They suggested that its effect on the choroidal circulation may be favorable as a glaucoma drug because it did not decrease blood flow.

On the other hand, there are not many reports about the long-term effect of unoprostone on human ocular circulation. Nishimura et al,[13] using color Doppler imaging, reported that the blood flow in the ophthalmic artery increased significantly in patients with normal-tension glaucoma after a 6-month treatment.

In this experiment, we used the laser speckle method that has been recently developed to provide a non-contact, two-dimensional analysis of capillary circulation in the retina, choroid, and ONH. Using this method in normal human eyes, Tamaki et al[14] reported that the coefficients of reproducibility at 1-minute interval measurements were 11.7% for NB_{ONH} and 8.7% for NB_{CHO} averaged over five pulses, while the 24-hour interval measurements were 13.0% for the former and 9.7% for the latter. These results indicate that microcirculation in ONH and choroid-retina can be measured with sufficient reproducibility by this method.

Although IOP did not change significantly on days 7 and 14 compared with day 0 in the treated eyes, it did decrease significantly on day 21. Because the subjects were healthy and their IOP before the experiment was low, IOP did not show significant change in treated eyes on days 7 and 14. The significant decrease on day 21 may be explained by the cumulative effect of unoprostone.

In the single-dose experiment by Kojima et al,[11] the NB_{ONH} did not change in the treated eyes but the NB_{CHO} increased significantly. Because ONH circulation does not change easily because of an autoregulatory mechanism, they suggested that the significant increase in NB_{CHO} may be due mainly to an increase in choroidal circulation caused by IOP reduction. On the other hand, in treated eyes on day 21, in the current study, NB_{ONH} showed a tendency to increase on day 21 before the instillation of unoprostone ($P = .087$), and it increased significantly after the instillation despite the autoregulatory mechanism. Because a significant effect on NB and IOP occurred only in the treated eyes, it is difficult to consider that the unoprostone was absorbed systemically, and then reached the posterior pole. If the long-term instillation causes more unoprostone to reach the posterior pole by the intraocular or periocular route and affect the ONH vessels directly, it may be possible to increase the ONH blood flow despite the autoregulatory mechanism.

NB_{ONH} at 4.5 hours after instillation on day 21 was higher than at the same time on day 0 in both treated and untreated eyes, although it was not significant. So we statistically compared the relative increase of NB (ΔNB) in treated and untreated eyes at the same time on day 0 and day 21 (Table 2), and no significant

Table 2. Increments of NB_{ONH} and NB_{CHO}*

	Before	4.5 hours
ΔNB_{ONH} (U)	0.28 ± 0.10	0.44 ± 0.12
ΔNB_{ONH} (P)	−0.12 ± 0.20	0.43 ± 0.23
ΔNB_{CHO} (U)	0.51 ± 0.21	0.88 ± 0.24†
ΔNB_{CHO} (P)	0.20 ± 0.24	0.39 ± 0.20†

*NB_{ONH}: normalized blur in optic nerve head, NB_{CHO}: normalized blur in middle of choroid retina. Unoprostone (U) and placebo (P) indicate increments of NB at same time on day 0 and day 21 in treated and untreated eyes, respectively. Data are expressed as mean ± SE (N = 11).

†$P < .05$ (paired t-test).

Table 3. Changes in Parameters*

	Before	4.5 Hours
Control experiment (day 0)		
BP_m (mm Hg)	90.1 ± 12.5	85.6 ± 11.1
Pulse rate (/min)	65.6 ± 8.1	70.8 ± 9.4
OPP_u (mm Hg)	44.5 ± 8.9	43.1 ± 7.5
OPP_p (mm Hg)	44.0 ± 8.7	42.9 ± 7.4
Unoprostone experiment (day 21)		
BP_m (mm Hg)	88.1 ± 10.9	83.0 ± 11.6
Pulse rate (/min)	67.0 ± 7.3	70.1 ± 10.7
OPP_u (mm Hg)	45.4 ± 7.4	43.2 ± 8.6
OPP_p (mm Hg)	43.9 ± 7.7	41.7 ± 8.7

*BP_m: mean blood pressure, OPP_U: ocular perfusion pressure in unoprostone-treated eyes, OPP_p: ocular perfusion pressure in placebo (control) eyes. Data are expressed as mean ± SD (N = 11).

Table 4. Correlations Between Parameters of Day 0 and Day 21 in Treated Eyes*

	Correlation Coefficient
Before instillation	
NB_{ONH} and OPP	0.218
NB_{ONH} and IOP	−0.045
NB_{CHO} and OPP	0.636 ($P < .01$)
NB_{CHO} and IOP	−0.616 ($P < .01$)
After instillation	
NB_{ONH} and OPP	0.228
NB_{ONH} and IOP	−0.243
NB_{CHO} and OPP	0.220
NB_{CHO} and IOP	−0.111

*NB_{ONH}: normalized blur in optic nerve head, NB_{CHO}: normalized blur in middle of choroid-retina (N = 11).

difference was observed in ΔNB_{ONH} before($P = .064$) and at 4.5 hours after instillation ($P = .971$). This suggests that the increase in NB_{ONH} in both eyes might be produced by causes other than the drug effect.

NB_{CHO} in the treated eyes on day 21 increased significantly both before and after the instillation, and the NB_{CHO} in the untreated eyes also increased although it was not significant. As ΔNB_{CHO} in the treated and untreated eyes differed significantly at 4.5 hours (Table 2), the increase in NB_{CHO} might have resulted from the effect of unoprostone itself.

Tissue blood flow is considered to be proportional to ocular perfusion pressure/resistance (OPP/R), where R equals the vascular resistance. Two factors may explain the increase in tissue blood flow: an increase in OPP or a decrease in vascular resistance. In an animal study, Ogo[8] found that administration of unoprostone increased choroidal circulation at constant OPP, and they suggested that an increase in blood flow was due to a reduction in choroidal vascular resistance. In our experiment, in the treated eyes before instillations, significant correlations were found between the relative changes in NB_{CHO} and OPP as well as between the NB_{CHO} and IOP (Table 4). These results suggest that the increase in NB_{CHO} on day 21 before the instillation may be mainly due to an increase in choroidal circulation caused by IOP reduction. On the other hand, OPP did not change significantly (Table 3) although NB_{CHO} increased the most at 4.5 hours after the instillation in the treated eyes on day 21. In addition, there was no correlation between the relative changes in NB_{CHO} and OPP, and those in NB_{CHO} and IOP (Table 4). These results suggest that the reduction in vascular resistance caused by unoprostone treatment may be the reason for the increase in NB_{CHO} at 4.5 hours after the instillation on day 21.

There is a possibility that unoprostone, a $PG_{F2\alpha}$-related compound, affects ONH and choroid-retinal vessels through unknown PG receptors or through nitric oxide which is regarded as a vasodilating factor,[17] but the exact mechanism needs further investigation.

Conclusions

The results of the present study suggest that long-term application of unoprostone increases the blood flow in the human ocular fundus.

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