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Research Article

Optic Nerve Head Blood Flow Changes Induced by Ripasudil Added to Prostaglandin Analogues in Primary Open Angle Glaucoma

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Abstract

Purpose: To evaluate the effect of topical ripasudil, a Rho-associated-kinase inhibitor, added to prostaglandin analogue eye drops on optic nerve head (ONH) blood flow in patients with primary open angle glaucoma (POAG).

Subjects and Methods: Subjects comprised 12 eyes from 12 patients with POAG who had been using a topical prostaglandin analogue for more than 3 months. ONH blood flow, intraocular pressure (IOP), blood pressure and pulse rate were measured before and 2 months after the treatment with a prostaglandin analogue and 0.4% ripasudil or 0.5% timolol in a randomized crossover study using the envelope method. ONH rim blood flow was assessed using laser speckle flowgraphy.

Results: The addition of 0.4% ripasudil or 0.5% timolol to the prostaglandin analogue significantly decreased IOP by the same degree. There was no correlation between changes of ocular perfusion pressure and ONH blood flow. Analysis of blood flow in 4 sections of the ONH rim showed that the combined treatment with a prostaglandin analogue and ripasudil, but not timolol, significantly increased ONH blood flow in all sections except for the temporal section.

Conclusions: The present study indicates that short-term treatment with ripasudil, added to a prostaglandin analogue, increases ONH blood flow probably due to its vasodilator action.

Keywords: *Optic Nerve Head; Blood Flow; Laser Speckle Flowgraphy; Ripasudil; Prostaglandin Analogue; Timolol*

Abbreviations

IOP: Intraocular Pressure; ROCK: Rho-Associated Kinase; ONH: Optic Nerve Head; POAG: Primary Open Angle Glaucoma; LSFG: Laser Speckle Flowgraphy; MBR: Mean Blur Rate; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; OPP: Ocular Perfusion Pressure

Introduction

Glaucoma is a multifactorial degenerative optic neuropathy that may progress at variable rates and is the second leading cause of blindness worldwide [1]. There have been many reports suggesting a role of ocular blood flow in the pathology of glaucoma in addition to the role of intraocular pressure (IOP) [2-7]. Recent progress has made it possible to measure ocular blood flow using reliable methods. Major methods for ocular blood flow measurement include color Doppler imaging, laser speckle flowgraphy (LSFG), and optic coherent tomography-based microangiography [8,9].

A Rho-associated kinase (ROCK) inhibitor, ripasudil, was approved in Japan for the treatment of glaucoma and ocular hypertension in September 2014 as the first anti-glaucoma agent in this drug category in the world [10,11]. While a number of reports have been published regarding the effects of various kinds of eye drops for glaucoma on ocular blood flow, there are few reports concerning the effects of ripasudil on ocular blood flow. Nakabayashi., *et al.* [12] reported that intravitreal injection of ripasudil increases retinal blood flow in cats.

To investigate the effects of a combined topical application of prostaglandin analogues and ripasudil on optic nerve head (ONH) blood flow in patients with glaucoma compared to those of β blockers, we conducted a crossover study.

Materials and Methods

We studied 12 eyes of 12 patients with primary open angle glaucoma (POAG) (6 males and 6 females; a mean age of 68.4 years) who had been using the topical therapy with prostaglandin analogue solutions (tafluprost: 7, latanoprost: 3, and travoprost: 2) in both eyes for more than 3 months. Exclusion criteria included smoking, severe cataract, retinal or optic nerve diseases other than glaucoma, glaucoma of advanced stage, high myopia (refractive error worse than -6.00 D), diabetes mellitus, and any systemic medication able to alter ocular blood flow, such as calcium channel blockers. All study conduct adhered to the tenets of the Declaration of Helsinki. This study protocol was reviewed and approved by the Institutional Review Board/Ethics Committee of Osaka Medical College. All participants provided informed consent to participate after the nature and possible consequences of study participation were explained.

The topical therapy with prostaglandin analogue solutions mentioned above was combined with either ripasudil solution (Glanatec 0.4%; Kowa Company, Ltd., Nagoya, Japan) or timolol solution (Timoptol 0.5%; Santen Pharmaceutical Co., Ltd., Osaka, Japan) in one of the eyes for 2 months each over the course of 4 months. The order of use of these concomitant drugs in the crossover study was determined using the envelope method, wherein a random assignment is written on pieces of paper for consecutive patients. The eye that received the combined therapy was also determined at random.

IOP, ONH blood flow, blood pressure, and pulse rate were measured before the combined therapy and after each 2-month treatment period with combined use of a prostaglandin analogue and ripasudil or timolol. The prostaglandin analogue was administered once per day (at night) while ripasudil and timolol were administered twice per day (in the morning and at night). All measurements were performed at almost the same time as the first measurement, which was adjusted to 2 - 3 hours after the morning application of the eye drops.

Subjects were instructed not to drink coffee or alcohol for one day prior to each measurement. IOP was measured by Goldmann applanation tonometer. Mydriasis was induced with 1 - 2 drops of topical 0.5% tropicamide (Mydrin-M ophthalmic solution; Santen Pharmaceutical Co., Ltd.) for measurement of ONH blood flow. ONH blood flow was measured in a sitting position. Each measurement was repeated three times using LSFG (LSFG-NAVI; SoftcareCo., Ltd., Fukuoka, Japan).

The principles of methods for determining ONH blood flow using LSFG have been previously described [13]. In the current study, mean blur rate (MBR) was used as an indicator of blood flow [14]. The MBR of the ONH tissue (MT) was automatically calculated using the LSFG Analyzer software (Ver. 7.0.26.0, Softcare, Ltd.). In this study, 4 divisions (superior, temporal, inferior, and nasal areas) of MT in the ONH rim were analyzed. As stated above, MT was measured three times at each time point examined. The average of these three measurements was used in data analyses. The patient treatment group was masked during data analysis.

Mean blood pressure (MBP) was calculated from systolic blood pressure (SBP) and diastolic blood pressure (DBP) using the following equation:

$$MBP=1/3\times(SBP - DBP) + DBP$$

Because blood pressure is pulsatile, ocular perfusion pressure (OPP) is defined as the weighted difference between MBP and IOP and was calculated using the following equation:

$$OPP = 2/3\times MBP - IOP$$

Results

There were no significant differences in sex and age distribution or IOP levels before the addition of ripasudil or timolol between the ripasudil-preceding and timolol-preceding groups (Table 1).

	Timolol- preceding group	Ripasudil-preceding group	P value
Number of cases	4	8	
Age (years)	69.5 ± 13.2	67.9 ± 8.7	0.40[a]
Sex (Male/Female)	2/2	4/4	0.70[b]
IOP before addition (mmHg)	13.8 ± 2.2	15.0 ± 2.5	0.20[a]

Table 1: *Age, sex, and IOP in each group.*

Data are presented as means ± standard deviations. [a] and [b] indicate unpaired t-test and Fisher's exact test, respectively.IOP, intraocular pressure.

IOP was reduced significantly 2 months following the initiation of combined therapy with ripasudil or timolol in both groups when compared to the IOP before the combined therapies. This reduction was maintained when the first combination was replaced with the other (Figure 1). Both combined therapies decreased IOP by the same amount (Table 2).

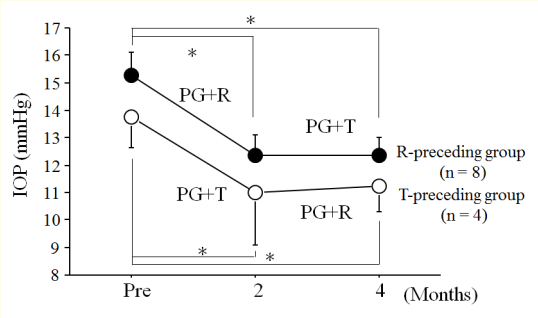

Figure 1: *Changes in intraocular pressure (IOP) in each group (mean ± standard error). *p < 0.05 (paired t-test). PG, R, and T represent prostaglandin analogues, ripasudil, and timolol, respectively.*

	PG alone	PG + R	PG + T
IOP (mmHg)	14.6 ± 2.4	12.0 ± 2.0**	11.9 ± 2.6**
MBP (mmHg)	92.1 ± 7.3	90.8 ± 6.2	90.3 ± 10.2
OPP (mmHg)	46.6 ± 5.1	48.4 ± 3.9	48.3 ± 6.9
Pulse rate (per min)	69.4 ± 8.7	66.8 ± 9.5	64.8 ± 7.1*

Table 2: *Changes in ocular and systemic parameters in all patients (n = 12).*

*Data are presented as means ± standard deviations. *and ** indicate statistical difference from PG alone levels (*p < 0.05, **P < 0.01, paired t-test). PG, prostaglandin analogues (tafluprost, latanoprost, or travoprost); R: ripasudil, T: timolol; IOP: intraocular pressure; MBP: mean blood pressure; OPP: ocular perfusion pressure.*

Figure 2 shows representative LSFG images illustrating changes in MT following both combined therapies. The MT increased when ripasudil was added to the prostaglandin analogue. However, the MT was unchanged following the timolol addition.

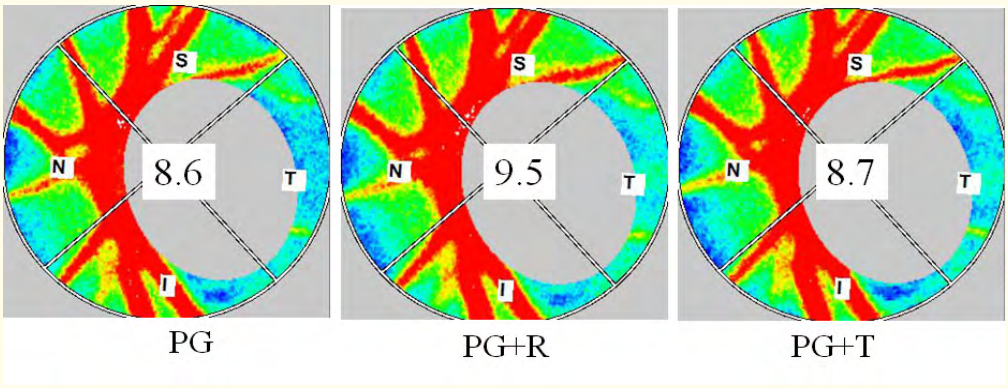

Figure 2: *A typical example of blood flow changes in the rim of the optic nerve head (ONH) shown by laser speckle flowgraphy following combined therapies in a 74-year-old patient. Red indicates higher ONH blood flow as represented by the mean blur rate (MBR), while blue indicates lower ONH blood flow. Tissue MBR (MT) is presented in the center of each figure panel.*

Average changes in ONH blood flow (MT) in both groups are shown in Figure 3. MT was significantly higher 2 months after the initiation of combined therapy with ripasudil than it was after combined timolol therapy and had a tendency to be higher than the previous levels in the ripasudil-preceding group. Although MT had a tendency for an increase after combined therapy with ripasudil also in the timolol-preceding group compared to the previous levels, there was no significant difference.

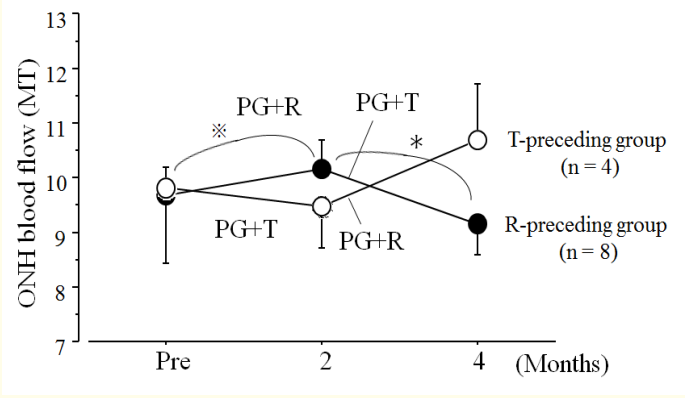

Figure 3: *Changes in the blood flow of optic nerve head (ONH) tissue (MT) in each group (mean ± standard error). $p < 0.05$, *$p < 0.1$ (paired t-test). PG, R, and T represent prostaglandin analogues, ripasudil, and timolol, respectively.*

Table 3 shows average changes in MT obtained from all subjects. The MT in all, superior, inferior, and nasal areas increased significantly 2 months after combined therapy with ripasudil compared to combined timolol therapy. Only the superior area had a higher MT after ripasudil addition than it did before the initiation of the combined therapies. On the other hand, the MT in the temporal area was unchanged following ripasudil addition. Combined therapy with timolol had no significant effect on the MT in any areas of the ONH. In eyes that were not treated with combined therapy, the MT was not changed significantly in all areas of the ONH (data not shown).

	All area	Superior area	Temporal area	Inferior area	Nasal area
PG alone	9.7 ± 1.8	11.1 ± 2.3	7.9 ± 1.8	9.7 ± 2.0	11.2 ± 2.7
PG + R	10.3 ± 1.6†	11.9 ± 2.1*,†	8.0 ± 2.0	10.3± 2.2†	12.1± 7.3†
PG + T	9.3 ± 1.5	10.6 ± 2.2	7.6 ± 1.3	9.2 ± 1.4	10.5 ± 2.6

Table 3: *Changes in mean blur rate in the rim area of optic nerve head tissue (MT).*

Data are presented as means ± standard deviations. *and† indicate statistical differences compared to PG alone and PG + T, respectively ($p < 0.05$, paired t-test). PG, prostaglandin analogues (tafluprost, latanoprost, or travoprost); R: ripasudil; T: timolol.

As shown in Table 2, MBP and OPP were unchanged after 2-month combined therapy with ripasudil or timolol. Pulse rate was reduced only following combined therapy with timolol.

Changes in ONH blood flow and OPP following ripasudil or timolol addition were not correlated with each other (simple regression analysis; ripasudil addition: p = 0.39, timolol addition: p = 0.70).

Discussion

To the best our knowledge, this is the first report on the effects of the combined application of a prostaglandin analogue and ripasudil on ONH blood flow. There was no significant difference between the IOP reductions induced by the additions of ripasudil vs. timolol to the prostaglandin analogue. However, ripasudil, but not timolol, significantly increased blood flow in all sections of the ONH rim except for the temporal section.

In the current study, a 2-month addition of ripasudil to prostaglandin analogues reduced IOP by about 2.6 mmHg. It has previously been reported that the mean IOP reduction from baseline following an 8-week addition of ripasudil to latanoprost was 3.2 mmHg, which was 1.4 mmHg higher than it was in the placebo group [15]. Our results describing the IOP-lowering effects of additive ripasudil are consistent with those of the above report.

Since changes in ONH blood flow and OPP after ripasudil addition were not correlated with each other in the present study, the increased blood flow might have been caused by a direct vasodilator effect of ripasudil, as it is a ROCK inhibitor. There have been several reports describing the vasodilator effects of other ROCK inhibitors in the eye. Topical administration of ripasudil or Y-39983, another ROCK inhibitor, was shown to increase blood flow to the ONH or retina in normal animals [12,16]. In fact, Nakabayashi., *et al.* [12] have reported that intravitreal injection of ripasudil increases retinal blood flow due to decreased vascular resistance downstream of the retinal arterioles. In addition, the vasodilator effects of fasudil, another ROCK inhibitor, on retinal arterioles in spontaneously hypertensive rats were reported to be greater than those in age-matched normotensive rats [17]. We have also reported that intravenous or topical application of fasudil prevents or improves ONH blood flow impairment, although it has no significant effects on normal ONH blood flow in rabbits [18]. It seems that ROCK inhibitors have more effects on ocular blood flow under pathological conditions than in normal states. A study on the effects of fasudil on forearm blood flow in humans suggested that a significant portion of ROCK-inhibitor-induced vasodilation is mediated by nitric oxide and that the constrictor response to endothelin (ET)-1 involves the activation of ROCK [19]. Another study reported that a ROCK inhibitor prevents endothelium-dependent constriction in the rat aorta [20]. Since there have been many reports on the implication of ET-1 and nitric oxide in the pathophysiology of glaucoma [21-24], our results showing that addition of ripasudil increases ONH blood flow in patients with glaucoma are consistent with previous research.

The addition of timolol had no significant effects on ONH blood flow in the current study. There have been several reports on the effects of timolol on ocular blood flow. Several reports have suggested that timolol might reduce ocular blood flow [25,26]. This may be explained by the vascular β_2-receptor blocking effect of this compound, which causes vasoconstriction [27]. On the other hand, other researchers have reported that a topical application of timolol causes no significant changes in ONH blood flow in normal subjects [28,29]. The role

of vascular β_2-receptors in the ONH microvasculature might not be significant enough to cause a vasoconstrictive effect at the concentrations that timolol is present at least following its topical application in humans.

There are only a few studies on the effects of combined therapy with a prostaglandin analogue and timolol on ocular blood flow. According to one of the reports, combined therapy with latanoprost and timolol increases ONH blood flow in monkeys [30]. Although prostaglandin $F_{2\alpha}$ usually constricts arteries, it functions as a vasodilator and increases blood flow in some tissues. Another study suggests that there are no significant changes in ONH blood flow following the same combined therapy for 3 months in patients with normal tension glaucoma [31]. Since we did not evaluate blood flow before the initiation of therapy with the prostaglandin analogues including latanoprost in the current study, we cannot determine whether prostaglandin analogues had any effects on ONH blood flow.

It should be noted that the present study has several limitations. First, we measured ONH blood flow only at one time point following the addition of each drug. The peak and trough of the effect of ripasudil on blood flow are still unknown. Second, we only evaluated changes in blood flow but did not assess the effects of ripasudil on visual function. The long-term efficacy of ripasudil on visual field defects in patients with glaucoma should be studied in the future.

Conclusion

The present study demonstrated that a short-term treatment of ripasudil added to a prostaglandin analogue increased ONH blood flow in glaucoma patients probably due to its vasodilator action.

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Conflict of Interest

The authors state that the manuscript has not been published previously, and they have no conflict of interest.

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