Effects of prostaglandin F<sub>2α</sub> analogues on endothelin-1-induced impairment of rabbit ocular blood flow: Comparison among tafluprost, travoprost, and latanoprost

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**Abstract**

We investigated the effects of prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>) analogues on the endothelin-1 (ET-1)-induced impairment of optic nerve head (ONH) blood flow and on ET-1-induced contraction in isolated ciliary artery segments. In male rabbits, one of four PGF<sub>2α</sub> analogues [0.0015% tafluprost, 0.0015% 15-hydroxyl tafluprost (15-OH tafluprost), 0.005% latanoprost, or 0.004% travoprost] was topically administered at various pretreatment times before intravitreal ET-1 injection. ONH blood flow was estimated by the laser speckle method, which expresses blood velocity as a quantitative index, the squared blur rate (SBR). SBR was measured just before (baseline value) and at 30, 60, and 120 min after ET-1 injection. SBR was significantly decreased from 4.47 ± 0.20 to 3.50 ± 0.10 (78.6 ± 2.4% of baseline) at 120 min after intravitreal ET-1 injection (5 pmol/eye). The ET-1-induced decrease was almost completely prevented by tafluprost and significantly inhibited by the other three analogues. The inhibitory effect lasted longest with tafluprost, as indicated by the effective pretreatment times (tafluprost: 90, 120, or 240 min; 15-OH tafluprost: 90, but not 120 or 240 min; latanoprost and travoprost: 120, but not 240 min). In vitro, the effects of PGF<sub>2α</sub> analogues on ET-1-induced contractions in male rabbit ciliary arteries were evaluated using an isometric tension recording system. Tafluprost, latanoprost, travoprost, and 15-OH tafluprost concentration-dependently relaxed the 10 nM ET-1-induced ciliary artery contraction. Improvement of the ocular circulation may be superior with tafluprost than with the other PGF<sub>2α</sub> analogues. The underlying mechanism may involve relaxation of ocular resistance vessels.

**Keywords:**
ciliary artery occlusion         
endothelin-1 (ET-1)                
tafluprost                        
latanoprost                       
travoprost

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1. Introduction

An elevated intraocular pressure (IOP) is known to be a major risk factor for the progression of glaucoma. However, recent clinical studies have suggested the additional involvement of impaired ocular blood flow (Grieshaber and Flammer, 2005), and indeed earlier reports had indicated that blood flow in the optic nerve head (ONH) was significantly lower in patients with open angle glaucoma (OAG) than in ocular hypertension patients or in normal volunteers (Michelson et al., 1996; Yamazaki and Hayamizu, 1995). In addition, it has been suggested that a decrease in ONH blood flow is linked to visual-field loss in glaucoma patients (Findl et al., 2000; Grunwald et al., 1998). On that basis, impaired blood circulation in the ONH may be one of the causes of glaucomatous optic neuropathy.

Although the precise mechanism responsible for glaucomatous ocular blood dysregulation remains unclear, it may involve an endogenous vasoactive peptide, endothelin-1 (ET-1). ET-1, which induces vasoconstriction mainly through the ETA receptor, is involved in the homeostatic regulation of the blood circulation, and intravenous administration of ET-1 reduces ocular blood flow in humans (Rubanyi and Polokoff, 1994; Polak et al., 2001). In glaucoma patients, the ET-1 concentration is elevated both in the plasma and in the aqueous humor (Cellini et al., 1997; Holló et al., 1998; Sugiyama et al., 1995; Tezel et al., 1997). In animal models, repeated intravitreal injection of ET-1 leads to a long-lasting reduction in ocular blood flow, resulting in decreases in the number of axons and in the content of neurofilament light chain (Sasaoka...
et al., 2006). These findings suggest that ET-1 may be causally involved in the pathogenesis of defective ocular blood flow in glaucoma patients.

The PGF$_{2\alpha}$ analogues latanoprost, travoprost, and tafluprost are widely used as IOP-reducing agents in glaucoma therapy. The effects of such PGF$_{2\alpha}$ analogues on the ocular blood circulation have been evaluated by several methods both in humans and in animal models. For example, it has been found that topical administration of latanoprost increases ONH blood flow in humans independently of its IOP-lowering effect (Ishii et al., 2001; Tamaki et al., 2001), and that topical administration of travoprost increases ocular blood flow in both humans and animals (Inan et al., 2004; Ohashi et al., 2007). Tafluprost has a characteristic chemical structure that differs from those of other prostaglandins (instead of a hydroxyl group at the carbon 15 position, it has two fluorine atoms), and in animal models it increases ocular blood flow more potently, and in a more stable manner, than other PGF$_{2\alpha}$ analogues (Izumi et al., 2008; Akaishi et al., 2010).

The purpose of this study was to examine whether PGF$_{2\alpha}$ analogues might limit the ET-1-induced impairment of ocular blood flow. We evaluated the effects of tafluprost, 15-hydroxyl tafluprost (15-OH tafluprost), travoprost, and latanoprost (Fig. 1) on the ET-1-induced impairment of ONH blood flow using laser speckle flowmetry in conscious rabbits. We also estimated the effects of PGF$_{2\alpha}$ analogues on ET-1-induced vascular contraction in rabbit isolated ciliary arteries.

2. Materials and methods

2.1. Animals

Male Dutch and Albino rabbits ($n = 98, 2.0–3.0$ kg) were supplied by Biotech Co. Ltd. (Saga, Japan). They were housed under a 12-h light–dark cycle, and allowed free access to standard food and water. All efforts were made to minimize the number of animals and their suffering. To minimize the number of animals, the rabbits in a control group were used in more than one experiment, and drug-treated rabbits were reused in other experiments. All animal care and experimental procedures were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved both by the Institutional Animal Care and Use Committee of Santen Pharmaceutical Co. Ltd. and by the Animal Experiment Committee of Akita University.

2.2. Materials

Tafluprost ophthalmic solution (0.0015% Tapros$^b$) was supplied by Santen Pharmaceutical Co. Ltd. (Osaka, Japan). Travoprost ophthalmic solution (0.004% Travatan$^Z$) was purchased from Alcon Laboratories Inc. (Fort Worth, TX, U.S.A). Latanoprost ophthalmic solution (0.005% Xalatan$^b$) was purchased from Pfizer Inc. (New York, NY, U.S.A.). Endothelin-1 (Human) was purchased from Peptide Institute Inc. (Osaka, Japan). For the in vitro study, latanoprost and travoprost were purchased from Cayman Inc. (Ann Arbor, MI, U.S.A), while tafluprost and 15-OH tafluprost were provided by Santen Pharmaceutical Co. Ltd.

2.3. Administration of ET-1 and ophthalmic solutions

Fifty microliters of one of the ophthalmic solutions or saline were topically administered at 90, 120, or 240 min before the ET-1 injection in the left eye. ONH blood flow measurement and intraocular injection were carried out in the left eye with the rabbit in a holding box. Each rabbit was placed in a holding box before baseline measurements, and ET-1 injection was carried out after baseline measurements. The procedure for preparing ET-1 solution is described below. As supplied, each vial contained 0.11 mg of ET-1, and when dissolved in 0.44 mL of 0.1% aqueous acetic acid, this provided a $10^{-4}$ M solution. The ET-1 concentration was adjusted by further dilution with balanced saline solution. Twenty microliters of ET-1 solution or saline was intravitreally injected into the posterior vitreous through a $30$-gauge needle under local anesthesia (induced using 0.4% oxibuprocaine hydrochloride; Benoxil$^b$, Santen Pharmaceutical Co. Ltd.). The pupil of the eye to be used was dilated with one drop of 0.4% tropicamide (Mydrin M$^b$; Santen Pharmaceutical Co. Ltd) to help us avoid hitting the lens or retina with the needle.

2.4. ONH blood flow measurement

Ocular blood flow is affected by the depth of anesthesia, and it is difficult to measure it accurately under anesthesia (Roth, 1992; Roth et al., 1993). Therefore, we and other laboratories have opted to measure ONH blood flow in rabbits in the conscious state (Goto et al., 2005; Sasaoka et al., 2006). In the present study, rabbits were placed in a holding box from baseline measurements to 120 min after ET-1 injection (about 140 min in total). No noteworthy abnormalities in the eyes or in the general health of the animals were observed when ONH blood flow measurements were made in the conscious state in this study or in a previous study (Akaishi et al., 2010). The pupil of the eye to be used for measurements was dilated with one drop of 0.4% tropicamide. Tissue blood flow in ONH was measured using laser speckle flowgraphy (Kyushu Institute of Technology, Iizuka, Japan). The detailed principle of measurement has been described elsewhere (Tamaki et al., 1995). ONH blood flow was measured in a surface vessels-free ONH tissue area of $1.7 \times 1.7$ mm ($100 \times 100$ pixels), measurements being made in the same area in a given animal throughout the experiment. The squared blur rate (SBR) value, a quantitative index of relative blood velocity, was calculated via a hardware-analyzed board for each pixel. The SBR value for a given time-point was calculated as the average of five sequential measurements obtained at intervals of approximately 5 s. Such measurements were made just before the ET-1 injection and at 30, 60, and 120 min after injection. We denominated the SBR value obtained just before ET-1 injection as
the “baseline SBR value”. Values obtained at subsequent time-points are expressed as a percentage of that baseline SBR value.

2.5. Isometric tension recording

In the current in vitro experiments, we used 6 rabbits. Ciliary arteries were isolated at four vessels per eye, and 46 vessels were used in the present in vitro study. Male Albino rabbits were anesthetized and euthanized by means of an intravenous injection of pentobarbital sodium (Nembutal Sodium®; Abbott Japan Co. Ltd.). The eyes were immediately enucleated, ensuring that the maximal length of optic nerve was removed, and placed in Krebs solution composed of the following (mM): NaCl 94.8, KCl 4.7, MgSO₄ 1.2, CaCl₂ 2.5, KH₂PO₄ 1.2, NaHCO₃ 25.0, glucose 11.7. This was oxygenated by bubbling with 95% O₂ and 5% CO₂. With the aid of connective tissue were carefully isolated from the optic nerve. A dissecting microscope, the ciliary artery and surrounding connective tissue were carefully isolated from the optic nerve. A vascular segment (150–300 μm in diameter, 1–2 mm in length) cut from the distal portion of the ciliary artery was immediately mounted in a chamber of a double Myograph System® (JP Trading, Denmark), which contained 10 ml Krebs solution. The temperature was maintained at 37 °C in the chamber. The Myograph System® directly determines vessel isometric tension and simultaneously transmits the data to a computer that displays the tension curves on a monitor. Details of this method of isometric tension recording have been published by Mulvany and Halpern (Mulvany and Halpern, 1976). To induce relaxation of vascular smooth muscle, carbachol (1 μM), a cholinergic agonist that acts on receptors in the endothelium, was added 20 min after contraction. This procedure confirmed the susceptibility of each contracted ciliary artery to cholinergic relaxation before we examined the effects of PGF₂α analogues. After an equilibration period, the contractions evoked by 10 nM ET-1 solution were measured at 20-min intervals to establish preparation viability and stability. The ability of tafluprost, latanoprost, travoprost, or 15-OH tafluprost (1–30 μM) to relax the isolated ciliary artery was determined in segments pre-contracted by 10 nM ET-1. Once the ET-1-induced contraction had been stable for 20 min, one of these four agents was applied every 10 min in a cumulative manner.

2.6. Statistical analysis

The SBR value obtained at a given time-point was expressed as a percentage of the baseline SBR value, and the mean ± S. E. M., with n representing the number of animals, was calculated for each group. The effects of intravitreal injections of ET-1 solution on SBR values were statistically analyzed using the Dunnett multiple-comparison test (ET-1-treated group vs. the control group) after two-way analysis of variance (ANOVA). The difference in time-matched SBR values (percentage of baseline) between the control group and a drug-treated group was analyzed using Student’s t-test or Aspin-Welch t-test. P < 0.05 was considered statistically significant. The percentage relaxation of the ciliary artery in the in vitro experiment was expressed as mean ± S.E.M., with n representing the number of vessels.

3. Results

3.1. Effect of intravitreal injection of ET-1 on ONH blood flow in rabbits

Intravitreal injection of ET-1 solution induced a reduction in ONH blood flow in rabbits (Fig. 2). The baseline SBR values were 4.17 ± 0.34 (control group, n = 4), 4.76 ± 0.42 (2.5 pmol/eye ET-1-injected group, n = 4), and 4.47 ± 0.20 (5 pmol/eye ET-1-injected group, n = 4), and there was no significant difference among the groups (Dunnett multiple-comparison test after two-way ANOVA). The SBR value was 4.61 ± 0.45 (96.7 ± 2.6% of baseline SBR value) at 120 min after the 2.5 pmol/eye ET-1 injection (not significantly different vs. time-matched control group, Dunnett multiple-comparison test after two-way ANOVA). Following intravitreal injection of 5 pmol/eye ET-1, the SBR value was 3.50 ± 0.10 (78.6 ± 2.4% of baseline SBR value) at 120 min after ET-1 injection (P < 0.01 vs. time-matched control group, Dunnett multiple-comparison test after two-way ANOVA). Therefore, ET-1 was given at 5 pmol/eye in the following examinations.

3.2. Effects of PGF₂α analogues on ET-1-induced decrease in ONH blood flow

The effects of tafluprost and 15-OH tafluprost on the ET-1-induced decrease in ONH blood flow are shown in Fig. 3. The baseline SBR value was not significantly different between any drug-treated group and its control group (data not shown). Intravitreal injection of 5 pmol/eye ET-1 decreased the SBR value to approximately 80% of the baseline SBR value at 120 min after the ET-1 injection. Pretreatment by tafluprost, latanoprost, travoprost, or 15-OH tafluprost (1–30 μM) to relax the isolated ciliary artery was determined in segments pre-contracted by 10 nM ET-1. Once the ET-1-induced contraction had been stable for 20 min, one of these four agents was applied every 10 min in a cumulative manner.

The effects of 15-OH tafluprost at 90 min after the ET-1 injection led to a significant suppression of the ET-1-induced reduction in the SBR value, although such pretreatment at 120 or 240 min before the ET-1 injection did not. A summary of the effects of the four PGF₂α analogues, as measured at 120 min after ET-1 injection, is shown in Fig. 5. Intravitreal injection of 5 pmol/eye ET-1 reduced the SBR value to approximately 80% of the baseline value at 120 min after the ET-1 injection in each evaluation. Drug pretreatment at 120 min before
the ET-1 injection produced the following results: (a) tafluprost and travoprost almost completely prevented the ET-1-induced decrease in ONH blood flow, (b) latanoprost tended to inhibit the ET-1-induced decrease and (c) 15-OH tafluprost had no effect. On the other hand, when drug pretreatment was given at 240 min before the ET-1 injection only tafluprost had a significant effect on the ET-1-induced decrease in ONH blood flow.

3.3. Effects of PGF2α analogues on ET-1-induced contraction of rabbit isolated ciliary artery

To help clarify the effects of PGF2α analogues on the ocular circulation, we evaluated the effects of tafluprost, latanoprost, travoprost, and 15-OH tafluprost on the ET-1-induced contraction of rabbit ciliary arteries in vitro. Treatment with 10 nM ET-1 elicited contraction, and exogenous tafluprost, latanoprost, travoprost, and 15-OH tafluprost each evoked a concentration-dependent relaxation on that contraction (Fig. 6). The percentage relaxations achieved at 10 μM of each of the PGF2α analogues were: 27.8 ± 5.0% (mean ± S.E.M.) (tafluprost, n = 11), 11.4 ± 4.0% (latanoprost, n = 13), 15.2 ± 5.6% (travoprost, n = 10), and −2.1 ± 5.5% (15-OH tafluprost, n = 12). The percentage relaxations achieved at 30 μM of each PGF2α analogues were: 70.7 ± 4.5% (tafluprost, n = 11), 41.9 ± 4.5% (latanoprost, n = 13), 51.7 ± 8.3% (travoprost, n = 10), and 25.9 ± 6.4% (15-OH tafluprost, n = 12).

4. Discussion

Previous experiments have revealed that topical administration of PGF2α analogues increases ONH blood flow in the rabbit (Akaishi et al., 2010; Ishii et al., 2001; Ohashi et al., 2007), and that tafluprost and latanoprost within the range 10^{-4} M – 10^{-6} M induce concentration-dependent relaxation of rabbit ciliary arteries pre-contracted with a high-K solution (Dong et al., 2008; Ishikawa et al., 2002). These results suggested that PGF2α analogues might be able to improve an impaired ONH blood flow. In the present study, we evaluated the effects of several PGF2α analogues on the ET-1-induced impairment of ONH blood flow in vivo and on ET-1-induced vascular contraction in vitro. In rabbits, intravitreal injection of 5 pmol/eye decreased ONH blood flow (i.e., SBR value), and pretreatment with any one of four PGF2α analogues inhibited this response. In addition, at a concentration-range selected from within that mentioned above, each of the four PGF2α analogues tested concentration-dependently relaxed the ET-1-induced contraction of the rabbit ciliary artery in vitro. In our previous studies, neither tafluprost alone nor latanoprost alone had
any effect on the mechanical properties of the rabbit ciliary artery at the concentrations employed in the present in vitro experiment (Ishikawa et al., 2002; Dong et al., 2008). These findings suggest that pretreatment with PGF2α analogues inhibits the ET-1-induced impairment of ONH blood flow via a mechanism that may involve relaxation of ocular arterial vessels.

Fluorine has become a widespread and important drug component, and its introduction may be expected to enhance the physicochemical properties and the adsorption, distribution, metabolism, and excretion properties of a given compound (Müller et al., 2007). Among the PGF2α analogues, tafluprost has a unique chemical structure. It has long been believed that the 15-hydroxyl group is essential for the biological activities of prostaglandins (Stjernschantz and Resul, 1992; Anderson et al., 1999), and indeed latanoprost and travoprost each have a hydroxyl group at the carbon 15 position. Tafluprost, the first PGF2α analogue to defy this commonly held belief, has di-fluorine at the carbon 15 position. In the present study, we found that tafluprost exerted an inhibitory effect on the ET-1-induced impairment of ONH blood flow that was more prolonged than those of 15-OH tafluprost, travoprost, and latanoprosten. The explanation for this is uncertain. However, the vasorelaxant effects of latanoprost and travoprost were slightly weaker than that of tafluprost in vitro, and in ophthalmic solutions the concentrations of latanoprost and travoprost are each three times higher than that of tafluprost. Therefore, it is assumed that tafluprost ophthalmic solution would not have a greater vasorelaxant effect than the other two PGF2α analogues in vivo, implying that tafluprost’s prolonged action is probably not due to its vasorelaxant effect. We speculate that metabolic stability, derived from the presence of di-fluorine at the carbon 15 position, contributed to the longer-lasting nature of tafluprost’s inhibitory effect in the present in vivo study. Generally, oxidation of the 15-hydroxyl group is one of the major metabolic pathways for prostaglandins (Keirse and Turnbull, 1975; Okita and Okita, 1996), and this leads to a marked loss of their biological activities. Oxidation of this 15-hydroxyl group is catalyzed by 15-hydroxy prostaglandin dehydrogenase (15-PGDH), and 15-PGDH is expressed in ONH and in red blood cells (Fujimori et al., 2002; Kaplan et al., 1975). The common technical document for travoprost indicates that ketonization of the 15-hydroxyl group is revealed by pharmacokinetic analysis, and it has been assumed that this ketonization causes loss of activity in the eye. In contrast, in the case of tafluprost, Fukano et al. reported that ketonization of the carbon 15 position was not detected in the eye in their pharmacokinetic analysis, and they suggested that there would be a prolonged pharmacological efficacy of tafluprost in the eye due to this metabolic stability (Fukano and Kawazu, 2009).

In the present study, tafluprost had a longer-lasting inhibitory effect on the ET-1-induced impairment of ONH blood flow than the three 15-OH-type PGF2α analogues tested (15-OH tafluprost, travoprost, and latanoprost). Our in vivo results are consistent with the di-fluorine at the carbon 15 position making tafluprost resistant to oxidation by the

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**Fig. 4.** Effects of travoprost and latanoprost on ET-1-induced impairment of ONH blood flow in rabbits. ET-1 solution (5 pmol/eye) was injected intravitreally. A test drug was topically administered at 120 or 240 min before the ET-1 injection. (A) (B) Effects of travoprost pretreatment on ET-1-induced decrease in ONH blood flow. (C) (D) Effects of latanoprost pretreatment on ET-1-induced decrease in ONH blood flow. Data are expressed as mean ± S. E. M. (n = 4 to 6). *P < 0.05, **P < 0.01 vs. time-matched control group (Student t-test).

**Fig. 5.** Summary of effects of PGF2α analogues on ET-1-induced impairment of ONH blood flow (measured at 120 min after ET-1 injection). Each drug was topically instilled at 120 or 240 min before ET-1 injection. The data are expressed as mean ± S.E.M. (n = 4 to 6).
15-PGDH present in ONH and blood, and therefore making its observed inhibitory effect more prolonged than those of the 15-OH-type PGF2α analogues. An investigation of the mechanism underlying tafufroprost’s prolonged action will need to await a future application of our in vitro experiment system.

The mechanism underlying the “anti-endothelin” action of PGF2α analogues has not yet been clarified. ET-1 binds to its receptor (typically the ET₁ receptor) on vascular smooth muscle cells (VSMCs), and this leads to an increase in the intracellular free Ca²⁺ concentration ([Ca²⁺]ᵢ) (Rubanyi and Polokoff, 1994). Among the possible mechanisms for the anti-endothelin action of PGF2α analogues are: (I) a direct antagonistic effect on the ET₁ receptor, (II) an increase in ocular perfusion pressure (OPP) following a change in IOP or blood pressure, (III) increased production of endothelium-derived vasodilators, or (IV) a direct inhibitory effect on the increase in [Ca²⁺]ᵢ. According to published (and some unpublished) data: (a) tafufroprost does not show an affinity for the ET₁ receptor (Takagi et al., 2004), (b) topical administration of tafufropost, latanoprost, or travoprost does not affect IOP in the rabbit (Akaishi et al., 2010; Ohashi et al., 2007; Orihashi et al., 2005; Taniguchi et al., 1997), (c) blood pressure is not changed by topical administration of travoprost in the rabbit (Ohashi et al., 2007) or by topical administration of tafufropost or latanoprost in the rabbit (our unpublished experiment), and (d) both tafufropost and latanoprost relax isolated rabbit ciliary artery segments precontracted with a high-K solution, with the amplitude of the relaxation being unchanged by L-NAME, indomethacin, or denudation of the vascular endothelium (Ishikawa et al., 2002; Dong et al., 2008). Therefore, it is unlikely that the anti-endothelin relaxation effects of PGF2α analogues are dependent on mechanisms (I), (II), or (III) above. It is generally accepted that the major part of the ET-1-induced sustained vascular contraction and increases in [Ca²⁺]ᵢ in VSMCs requires a persistent entry of extracellular Ca²⁺ through various types of Ca²⁺ channel (Rubanyi and Polokoff, 1994). In our previous report, we suggested the participation of a capacitative Ca²⁺-entry channel in the mechanism underlying the vascular smooth muscle relaxation induced by tafufropost (since tafufropost inhibited capacitative Ca²⁺ entry) (Dong et al., 2008). It is generally accepted that the vascular contraction and increase in [Ca²⁺]ᵢ induced by ET-1 depend partially on Ca²⁺ entry through capacitative Ca²⁺ channels (Komuro et al., 1997; Zhang et al., 1998), and it is possible that the anti-endothelin action of PGF2α analogues is due, at least in part, to inhibition of ET-1-induced capacitative Ca²⁺ entry.

The present study suggests that PGF2α analogues improve ocular circulation in glaucoma patients. However, direct extrapolation of the current results to humans may be difficult because vascular responses to PGF2α analogues and regulation of vascular tone vary among species. In vitro, a myograph system is generally used for investigations of the mechanisms of action of vasoactive agents, but it has unavoidable limitations. Yu et al. pointed out in their review on the applications of such a system in ophthalmic research (Yu et al., 2003) that with an in vitro myograph system: (1) it is impossible to recreate exactly the in vivo environment, (2) there is an absence of perfusion blood flow, and (3) neural control mechanisms are lost. Therefore, we considered it too difficult to discuss the relation between the concentrations/doses used in in vitro and in vivo experiments. We speculate that PGF2α analogues may inhibit ET-1-induced contraction not only of ciliary arteries, but also of other ocular vessels such as retinal artery or retinal microvessels. In the present study, we employed the rabbit ciliary artery because the principal blood supply of the rabbit ONH is derived from the ciliary arteries and increase in ocular perfusion pressure (OPP) following a change in IOP or blood pressure, (III) increased production of endothelium-derived vasodilators, or (IV) a direct inhibitory effect on the increase in [Ca²⁺]ᵢ. According to published (and some unpublished) data: (a) tafufropost does not show an affinity for the ET₁ receptor (Takagi et al., 2004), (b) topical administration of tafufropost, latanoprost, or travoprost does not affect IOP in the rabbit (Akaishi et al., 2010; Ohashi et al., 2007; Orihashi et al., 2005; Taniguchi et al., 1997), (c) blood pressure is not changed by topical administration of travoprost in the rabbit (Ohashi et al., 2007) or by topical administration of tafufropost or latanoprost in the rabbit (our unpublished experiment), and (d) both tafufropost and latanoprost relax isolated rabbit ciliary artery segments precontracted with a high-K solution, with the amplitude of the relaxation being unchanged by L-NAME, indomethacin, or denudation of the vascular endothelium (Ishikawa et al., 2002; Dong et al., 2008). Therefore, it is unlikely that the anti-endothelin relaxation effects of PGF2α analogues are dependent on mechanisms (I), (II), or (III) above. It is generally accepted that the major part of the ET-1-induced sustained vascular contraction and increases in [Ca²⁺]ᵢ in VSMCs requires a persistent entry of extracellular Ca²⁺ through various types of Ca²⁺ channel (Rubanyi and Polokoff, 1994). In our previous report, we suggested the participation of a capacitative Ca²⁺-entry channel in the mechanism underlying the vascular smooth muscle relaxation induced by tafufropost (since tafufropost inhibited capacitative Ca²⁺ entry) (Dong et al., 2008). It is generally accepted that the vascular contraction and increase in [Ca²⁺]ᵢ induced by ET-1 depend partially on Ca²⁺ entry through capacitative Ca²⁺ channels (Komuro et al., 1997; Zhang et al., 1998), and it is possible that the anti-endothelin action of PGF2α analogues is due, at least in part, to inhibition of ET-1-induced capacitative Ca²⁺ entry.

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In conclusion, the present study is the first to report (a) that topical administration of PGF2α analogues can inhibit the ET-1-induced decrease in ONH blood flow in conscious rabbits and (b) that PGF2α analogues relax rabbit isolated ciliary artery segments precontracted by ET-1. Since the inhibitory effect of tafufropost on the ET-1-induced decrease in ONH blood flow was more prolonged than those of 15-OH tafufropost, travoprost, and latanoprost, the presence of di-fluorine at the carbon 15 position may contribute to the longer-lasting nature of this inhibitory effect of tafufropost. These results suggest that as regards ocular circulation improvement, tafufropost may be superior to other PGF2α analogues.

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