# IOP-Lowering Effect of ONO-9054, A Novel Dual Agonist of Prostanoid EP3 and FP Receptors, in Monkeys

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**Purpose.** The purpose of this study was to determine whether a better IOP reduction can be observed in conscious, normotensive monkeys treated with ONO-9054, a novel dual EP3 and FP receptor agonist, compared with prostaglandin $F_{2}\alpha$ analogs.

**Methods.** The binding affinities and agonistic activities of ONO-AG-367, a carboxylic acid of ONO-9054, to prostanoid receptors were assessed. The IOP-lowering effect of ONO-9054 in monkeys was analyzed after a single (0.3, 3, or 30 μg/mL) or 7-day repeated (30 μg/mL, every day) topical ocular administration. Ophthalmologic and histopathologic evaluations of the eye were performed after 4-week ocular administration of ONO-9054 (30 μg/mL, twice a day) in monkeys.

**Results.** The ONO-AG-367 exhibited high affinity for both EP3 and FP receptors and potent agonist activity, with $EC_{50}$ values of 28.6 nM for the EP3 receptor and 22.3 nM for the FP receptor. Single and repeated topical ocular administration of ONO-9054 caused IOP reductions in normotensive monkeys. The maximum IOP reductions on day 7 observed with ONO-9054 (7.3 ± 0.8 mm Hg) were significantly greater than those observed with latanoprost (50 μg/mL, 4.9 ± 0.4 mm Hg) or travoprost (40 μg/mL, 5.1 ± 0.6 mm Hg). In ophthalmologic and histopathologic evaluations, slight and transient mydriasis was occasionally observed and no histopathologic lesions attributable to ONO-9054 were noted.

**Conclusions.** A more profound and longer-lasting reduction in IOP in normotensive monkeys can be observed with ONO-9054, which simultaneously stimulates both EP3 and FP receptors, compared with prostaglandin analogs.

Keywords: prostaglandin, prostanoid receptor, intraocular pressure, EP3, FP

Glaucoma is the second leading cause of irreversible blindness among adults worldwide.[1–3] Studies have confirmed that elevated IOP is the main risk factor for the progression of glaucoma, and medical therapy aimed at reducing IOP has been demonstrated as lowering risks for further progressive damage to visual fields.[4] The IOP can be lowered with medical therapy, laser treatment, or surgery (individually, or in combination); however, in most instances, topical medications constitute the initial therapy. Current trends show that once-daily topical prostaglandin analogs (PGAs) are becoming the first choice for glaucoma therapy.

Prostaglandin analogs, such as latanoprost and travoprost, are thought to lower IOP mainly via the prostanoid FP receptor, which is a receptor for prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). On the other hand, a novel mechanism of action for PGAs via the prostanoid EP3 receptor has been reported recently.[5] The prostanoid EP3 receptor is one of four receptors identified for prostaglandin $E_2$ ($PGE_2$). Prostanoid EP3 receptors have distinct cellular distributions in tissues involved in the uveoscleral and conventional pathways that regulate IOP.[6] Although the IOP-lowering effect of $PGE_2$ and its derivatives in humans[7] and animals[8] has been reported, there are no approved drugs for glaucoma therapy. In this study, we investigated whether compounds that simultaneously stimulate both prostanoid EP3 and FP receptors compared with PGAs would result in more profound IOP reductions in monkeys.

## Materials and Methods

### Materials

Ono Pharmaceutical Co., Ltd. (Osaka, Japan) synthesized ONO-9054 (Propan-2-yl 4-{(3S,5aR,6R,7R,8aS)-6-[(1E,3R)-4-(2,5-difluorophenoxy)-3-hydroxybut-1-en-1-yl]-7-hydroxyoctahydro-2H-cyclopenta[b]oxepin-3-yl}butanoate) and its biologically active free acid ONO-AG-367 (4-{(3S,5aR,6R,7R,8aS)-6-[(1E,3R)-4-(2,5-Difluorophenoxy)-3-hydroxybut-1-en-1-yl]-7-hydroxyoctahydro-2H-cyclopenta[b]oxepin-3-yl}butanoic acid) (Fig. 1).

Commercially available latanoprost (Xalatan Eye Drops 0.005%; Pfizer, New York, NY, USA) and travoprost (Travatan Z Ophthalmic Solution 0.004%; Alcon Japan, Tokyo, Japan) also were used in this study.

### Binding Affinity to Prostanoid Receptors

The binding affinities of ONO-9054 and ONO-AG-367 were evaluated in radiolabeled ligand-binding assays using membranes prepared from Chem-1 cells stably expressing human FP receptors and Chinese hamster ovary (CHO) cells stably expressing human EP1, human EP2, human $EP3_I$, human EP4, mouse IP, mouse TP, and mouse DP receptors. Membranes expressing these receptors were incubated with a fixed concentration of radiolabeled ligands ([$^3$H]-$PGE_2$ for the EP1,

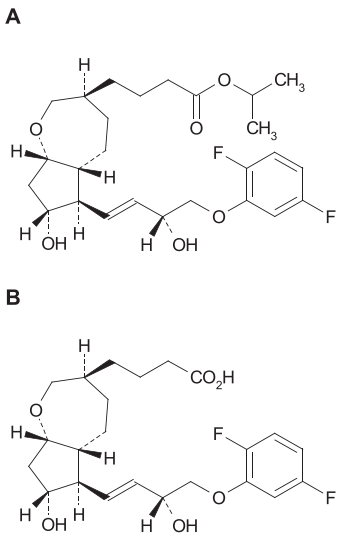


FIGURE 1. Structure formula. (**A**) ONO-9054. (**B**) ONO-AG-367.

EP2, EP3, and EP4 receptor assays; [$^3$H]-$PGF_{2\alpha}$ for the FP receptor assay; [$^3$H]-Iloprost for the IP receptor assay; [$^3$H]-SQ29548 for the TP receptor assay; and [$^3$H]-prostaglandin $D_2$ for the DP receptor assay) and the test compound in the following buffers: 10 mM $KH_2PO_4$-KOH buffer, pH 6.0, containing 1 mM EDTA, 10 mM $MgCl_2$, and 100 mM NaCl for the EP1, EP2, EP3, EP4, and FP receptor assays; 25 mM HEPES-NaOH buffer, pH 7.4, containing 1 mM EDTA, 5 mM $MgCl_2$, and 10 mM $MnCl_2$ for the DP receptor assay; 10 mM Tris-HCl buffer, pH 7.4, containing 100 mM NaCl for the TP receptor assay; and 50 mM Tris-HCl buffer, pH 7.4, containing 1 mM EDTA and 10 mM $MgCl_2$ for the IP receptor assay. After reaching equilibrium, the assays were terminated by rapid filtration through Whatman GF/B filters (Brandel Co., Gaithersburg, MD, USA). The filters were washed with the following ice-cold buffers: 10 mM $KH_2PO_4$-KOH buffer, pH 6.0, containing 100 mM NaCl for the EP1, EP2, EP3, EP4, and FP receptor assays; 10 mM Tris-HCl buffer, pH 7.4, containing 100 mM NaCl and 0.01 wt/vol% BSA for the DP receptor assay; and 10 mM Tris-HCl buffer, pH 7.4, containing 100 mM NaCl for the TP and IP receptor assays. The filters were then dried for 60 minutes at 60°C, and radioactivity on the filter was measured using a liquid scintillation counter (TRI-CARB2900TR; Perkin Elmer Japan, Kanagawa, Japan).

Nonspecific binding was defined by adding excess amounts of unlabeled ligands (unlabeled $PGE_2$ for the EP1, EP2, EP3, and EP4 receptor assays; unlabeled $PGD_2$ for the DP receptor assay; unlabeled SQ29548 for the TP receptor assay; unlabeled $PGF_{2\alpha}$ for the FP receptor assay; or unlabeled Iloprost for the IP receptor assay). Specific binding was calculated by subtracting nonspecific binding from total binding. The dissociation constant (Kd) and maximum specific binding (Bmax) were determined by nonlinear regression analysis. The Bmax was defined as 100%, and 50% inhibitory concentration ($IC_{50}$) values were calculated from inhibition rates (%) of the test compounds at each concentration. The $IC_{50}$ values were converted to an absolute inhibition constant Ki by using the Cheng-Prusoff equation.

## Prostanoid Receptor Agonist Activity

The agonist activities of ONO-9054 and ONO-AG-367 were evaluated using intracellular $Ca^{2+}$ signaling responses in Chem-1 cells expressing human FP receptors and CHO cells expressing human EP3 receptors. Cells were transferred at 1 $\times 10^4$ cells per well in 96-well plates and cultured for 2 days with MEM Eagle alpha modification containing 10% fetal bovine serum (10% FBS/αMEM) in an incubator (37°C, 5% $CO_2$, and 95% air). Cells were rinsed with phosphate buffer, and loaded with 5 μM Fura 2-AM in 10% FBS/αMEM containing 10 mM HEPES, 20 μM indomethacin, and 2.5 mM probenecid. After incubation for 1 hour, cells were rinsed twice with a buffer consisting of Hank's balanced salt solution, 0.1 wt/vol% BSA, 2 μM indomethacin, 2.5 mM probenecid, and 20 mM HEPES. The same buffer, but containing 1 wt/vol% BSA, was then added and cells were incubated in the dark at room temperature for 1 hour. After addition of the test compound, intracellular $Ca^{2+}$ concentration was measured as a fluorescence ratio of emission (340/380 nm) using a fluorescence drug screening system (FDSS-3000; Hamamatsu Photonics, Tokyo, Japan). Maximum increases of intracellular $Ca^{2+}$ concentrations with $PGE_2$ or $PGF_{2\alpha}$ treatment were defined as 100%, and 50% effective concentration ($EC_{50}$) values of test compounds were calculated from effective rates (%) at each concentration using the Sigmoid $E_{max}$ model.

## Animals

All experiments were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, the Partial Amendments to the Law for the Humane Treatment and Management of Animals (Law No. 68, Jun. 22, 2005, Japan), and the Guidance for Animal Care and Use (revised on Nov. 7, 2007), and in accordance with the protocol reviewed by the Institutional Animal Care and Use Committee of Ina Research, Inc., which is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International (accredited unit no. 001107). Cynomolgus monkeys (supplied by Scientific Primates Filipinas, Metro Manila, Philippines; SICONBREC, Rizal, Philippines; DEL MUNDO TRADING, Oriental Mindoro, Philippines; NAFO-VANNY, Dong Nai, Vietnam; and Yunnan National Laboratory Primate Center, Yunnan, China) were used in this study, and monkeys were acclimated to a light schedule of alternating 12-hour periods of light and darkness for at least 1 week before dosing.

## Preparation of Eye Drops

The ONO-9054 was dissolved in a vehicle containing 0.1 wt/vol% polysorbate 80, 0.3 wt/vol% sodium citrate, 0.8 wt/vol% sodium chloride, and 0.01 wt/vol% benzalkonium chloride (pH 6.4, 286 mOsm). Commercially available latanoprost and travoprost solutions were used in this study.

## Effect on IOP in Monkeys

Male cynomolgus monkeys between the ages of 6 and 14 years were used in this study. Monkeys had been trained to accept restraint, topical ocular administration of eye drops, and IOP measurement before this experiment. Although the monkeys were used repeatedly in this study, there was a washout period of a minimum of 7 days between cycles.

Each monkey was positioned in a seated posture in a custom-designed monkey chair with arms gently restrained, and a 30-μL volume of the drugs and vehicle were topically applied once into each eye of conscious monkeys. The IOPs in conscious seated monkeys were measured with an applanation pneumatonometer (Model 30 Classic; Reichert Technologies, Buffalo, NY, USA). The monkeys were distributed into treatment groups to ensure similar baseline IOP as far as possible.

**Table 1.** Binding Affinities (Ki) of ONO-9054 and ONO-AG-367 to Prostanoid Receptors

| | Ki, nM | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Compounds** | **EP1** | **EP2** | **EP3** | **EP4** | **FP** | **IP** | **TP** | **DP** |
| ONO-9054 | >10,000 | >10,000 | >10,000 | >10,000 | 16.8 (13.5–20.8) | >10,000 | >10,000 | >10,000 |
| ONO-AG-367 | 734 (424–1270) | >10,000 | 25.0 (20.8–30.1) | >10,000 | 0.727 (0.637–0.829) | >10,000 | >10,000 | >10,000 |

Ki is the dissociation constant of the compound for the receptor. The 95% confidence intervals are shown in parentheses.

In the single-dose study, the IOP-lowering effect of ONO-9054 at 0.009 μg, 0.09 μg, and 0.9 μg (0.3, 3, 30 μg/mL) was examined in conscious normotensive monkeys. The IOPs were measured before (baseline IOP) and at 4, 8, 12, 24, 48, and 72 hours after administration. The ONO-9054 was topically applied once into the left eye. Reduction of IOP for each group was expressed as the mean ± SEM ($n = 8$).

In the repeated-dose study, IOP-lowering effects of ONO-9054 (30 μg/mL), latanoprost (50 μg/mL), and travoprost (40 μg/mL) were examined in conscious normotensive monkeys. Drops were topically applied once daily into each eye every morning for 7 days. Four of the eight monkeys in each group received the drug in the left eye, and the other four received the drug in the right eye. The IOPs were measured just before (baseline IOP) and at 4, 8, 12, and 24 hours after administration on days 1, 4, and 7, as well as at 48 hours after the last administration. Reduction of IOP for each group was expressed as the mean ± SEM ($n = 8$).

### Assessment of Ocular Safety in Monkeys

Male and female cynomolgus monkeys at the age of 3 years were used in this study. Ophthalmologic (pupil size, macroscopic assessments of the anterior segment of the eyes) and histopathologic evaluation of the eyes and eyelids was performed, after administration of ONO-9054 at 30 μg/mL or vehicle twice daily with a 6-hour interval, in the left eye of six monkeys in each group (three of each sex per group) for 4 weeks. The ONO-9054 and vehicle (30 μL) were topically applied once into the left eyes in conscious monkeys, which were gently restrained by a technician. Measurements of pupil size under a constant illuminance (400–600 lux) were performed before the first administration and at 2, 4, 6 (before the second administration), and 24 hours after the first administration on days 1, 3, 7, 14, and 27. Dilation (mydriasis) of pupil size by 1.0 mm or more was noted in the left eye treated with ONO-9054 compared with the untreated eye (right eye). The anterior segment (cornea, conjunctiva, and iris) was macroscopically evaluated according to Draize's scale[9] before the first administration on day 1 and at 1 hour after the second administration on days 1, 3, 7, 14, 21, and 27. The cornea was evaluated for both degree of corneal opacity and area of the cornea in which opacity is involved. The iris was assessed for inflammation, iridal folds, congestion, swelling, circumcorneal injection, reaction to light, hemorrhage, and gross destruction. The conjunctiva was evaluated for degree of redness, swelling, and discharge.

### Data Analysis and Statistics

Data presented in the figures represent the mean ± SEM values. Data were analyzed using the SAS System, release 9.2 TS2M3 software (SAS Institute Japan, Tokyo, Japan) and its interlocked system, EXSAS, version 7.7.1 (CAC EXiCARE, Tokyo, Japan) and plotted as IOP (mm Hg) versus time (hours). Paired *t*-tests were performed to test significance of differences between baseline IOP and posttreatment IOP. To compare data points from different treatment groups, ANOVA and post hoc Dunnett's tests were used. The level of significance was $P$ less than 0.05.

## Results

### Binding Affinity to Prostanoid Receptors

Only ONO-9054 exhibited high affinity for the FP receptor and exhibited no affinity for any other prostanoid receptors. The Ki of ONO-9054 for the FP receptor was 16.8 nM and the Ki for the EP1, EP2, EP3, EP4, DP, IP, and TP receptors was greater than 10 μM. The ONO-AG-367 exhibited high affinity for both EP3 and FP receptors. The Ki of ONO-AG-367 for EP1, EP3, and FP receptors was 734, 25, and 0.727 nM, respectively, whereas the Ki for EP2, EP4, DP, IP, and TP receptors was greater than 10 μM (Table 1).

### Prostanoid Receptor Agonist Activity

The ONO-9054 exhibited low agonist activity with $EC_{50}$ values greater than 10 μM for the EP3 receptor and 3030 nM for the FP receptor, whereas the carboxylic acid ONO-AG-367 exhibited potent agonist activity with $EC_{50}$ values of 28.6 nM for the EP3 receptor and 22.3 nM for the FP receptor (Table 2).

### Effect on IOP in Monkeys

The IOP-lowering effect of ONO-9054 was investigated in normotensive monkeys after a single ocular administration with 0.3, 3, or 30 μg/mL ONO-9054. At concentrations of 0.3 μg/mL or more, ONO-9054 significantly lowered the IOP from baseline in a dose-dependent manner. The IOP reduction at 3 μg/mL was observed for up to 24 hours after administration and the IOP reduction at 30 μg/mL persisted for up to 48 hours after administration. Maximum IOP reductions with ONO-9054 were observed 12 hours after administration, with reductions at 3 and 30 μg/mL of 4.3 ± 0.2 and 5.9 ± 0.3 mm Hg, respectively. The most potent IOP reduction that was sustained for the longest period of time was observed with ONO-9054 at 30 μg/mL (Fig. 2). Individual IOP responses are provided in the Supplementary Material (Supplementary Table S1).

In the 7-day repeated-dose study, ONO-9054 (30 μg/mL), latanoprost (50 μg/mL), and travoprost (40 μg/mL) caused IOP reductions at 4, 8, 12, and 24 hours after administration on

**Table 2.** Agonist Potencies ($EC_{50}$) of ONO-9054 and ONO-AG-367 Against EP3 and FP Receptors

| | $EC_{50}$, nM | |
|---|---|---|
| **Compounds** | **EP3** | **FP** |
| ONO-9054 | >10,000 | 3030 (2580–3560) |
| ONO-AG-367 | 28.6 (25.5–32.0) | 22.3 (19.8–25.1) |

$EC_{50}$ is the half maximum effective concentration of compounds for the receptor. The 95% confidence intervals are shown in parentheses.

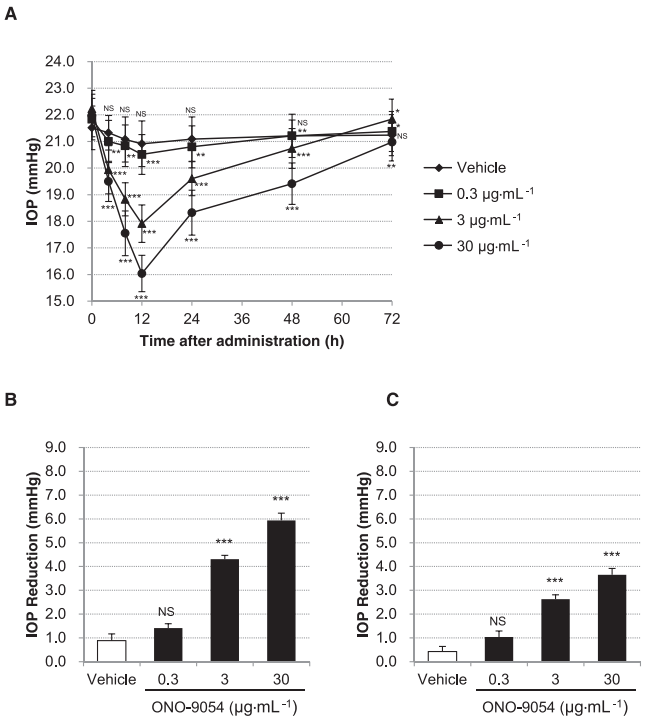


**Figure 2.** Intraocular pressure induced by a single topical ocular administration of ONO-9054 in normotensive monkeys. (**A**) Time course and dose-response. (**B**) Maximum IOP reduction. (**C**) IOP reduction at 24 hours. Values represent the mean ± SEM for eight animals. Significant differences between baseline IOP and posttreatment IOP by paired *t*-test or between groups by ANOVA and post hoc Dunnett's test. NS, not significant. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

days 1, 4, and 7. Maximum IOP reductions observed with ONO-9054 (5.8 ± 0.7, 6.6 ± 0.7, and 7.3 ± 0.8 mm Hg on days 1, 4, and 7, respectively) were greater than those observed with latanoprost (3.4 ± 0.6, 4.6 ± 0.5, and 4.9 ± 0.4 mm Hg) or travoprost (4.0 ± 0.5, 4.9 ± 0.5, and 5.1 ± 0.6 mm Hg) on the same days. Furthermore, ONO-9054 showed longer-lasting IOP-lowering effects after the last administration on day 7 (Fig. 3). Individual IOP responses are provided in the Supplementary Material (Supplementary Table S2).

Additionally, ocular pharmacokinetics were investigated after a topical ocular instillation of [3H]-ONO-9054 (4.8 μg per animal) in two monkeys. The analyses of metabolite profile demonstrated that the radioactivity reflected mainly the level of ONO-AG-367 in the aqueous humor (Supplementary Table S3).

## Assessment of Ocular Safety in Monkeys

In the 4-week repeated-dose toxicity study in monkeys, slight (1.0 mm) and transient mydriasis was occasionally observed in the eyes treated with ONO-9054; no abnormalities attributable to ONO-9054 were observed in Draize evaluations of anterior segments (cornea, conjunctivae, and iris) of the eyes or histopathology of the eyes and eyelids. Mydriasis was observed in only two of six animals at 2 hours after the first administration of ONO-9054 on day 1, which recovered by 4 hours after the first administration in all animals. No appreciable changes were noted in pupil size from day 2 and no abnormalities were observed in the lighting reflex during the appearance of mydriasis. There were no abnormalities in any of the vehicle-treated eyes (Table 3).

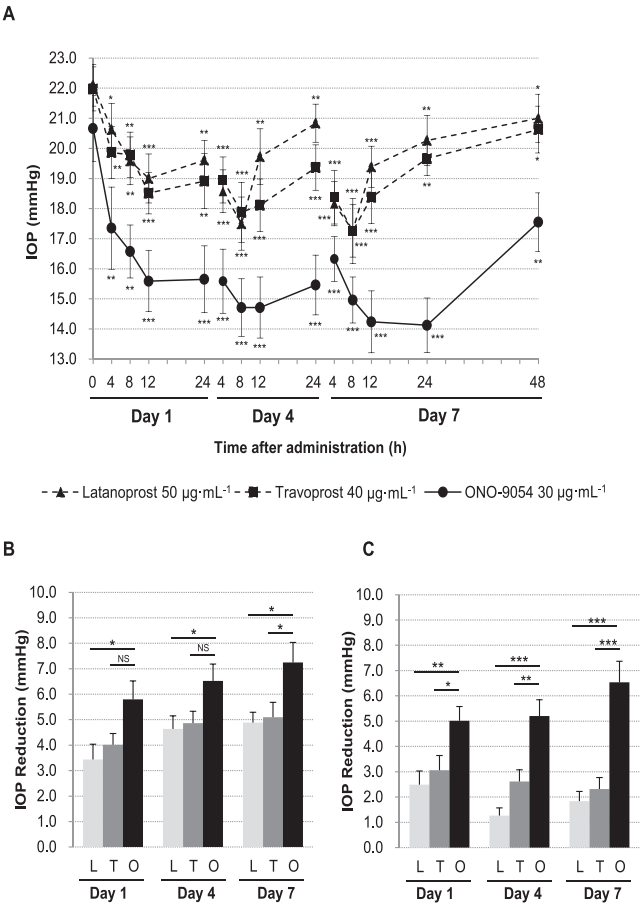


**Figure 3.** Intraocular pressure induced by repeated topical ocular administration of ONO-9054, latanoprost, or travoprost once daily for 7 days in normotensive monkeys. (**A**) Time course and dose-response. (**B**) Maximum IOP reduction. L, latanoprost 50 μg/mL; T, travoprost 40 μg/mL; O, ONO-9054 30 μg/mL. (**C**) Reduction in IOP at 24 hours. Values represent the mean ± SEM for eight animals. Significant differences between baseline IOP and posttreatment IOP by paired *t*-test or between groups by ANOVA and post hoc Dunnett's test. NS, not significant. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Additionally, a rabbit (New Zealand White; Kitayama Labes, Nagano, Japan) toxicity study using 50 μg/mL of ONO-9054 (instilled twice daily for 4 weeks) was conducted. Macroscopic observations of the anterior parts of the eyes revealed redness of the conjunctiva at 1 hour after the second instillation of the ONO-9054. There were no other findings suggestive of irritation in conjunctiva and cornea or iris. No total Draize's scale score exceeded 2.0 on day 1 (Supplementary Table S4).

**Table 3.** Ophthalmologic and Histopathologic Evaluation After Treatment of ONO-9054 in Monkeys

| | Vehicle | 30 μg/mL |
|---|---|---|
| No. of deaths | 0 | 0 |
| Ophthalmology | | |
| Pupil size (mydriasis)* | 0/6 | 2/6 |
| Draize score (cornea, conjunctiva, and iris)† | 0.0 | 0.0 |
| Histopathology | N | N |

N, no toxic histopathologic lesions.

* Values are expressed as the incidence (number of animals with at least 1.0-mm difference from untreated eye/examined).

† Values of Draize score as the mean.

## Discussion

The present study has demonstrated that the IOP-lowering effects of ONO-9054 were of great magnitude and longer-lasting than those observed with two PGAs, latanoprost and travoprost, in monkeys. The effects could be attributable to costimulation of EP3 and FP receptors, as ONO-9054, the isopropyl ester prodrug form of ONO-AG-367, had strong agonistic activity for both EP3 and FP receptors. Our results are consistent with the hypothesis that stimulation of these two receptors has more profound therapeutic effects than PGAs in glaucoma. It should be also noted that ONO-9054 showed no abnormalities of anterior portions of the eyes or histopathology of the eyes and eyelids except for mydriasis.

Prostaglandin analogs, such as latanoprost and travoprost, have been used as ocular hypotensive agents for more than a decade and are generally considered to be reasonable first-line treatments for glaucoma. The interaction of PGAs with the FP receptor is clearly crucial for their effect on IOP, as demonstrated by their lack of effect on IOP in FP receptor–deficient mice.[5,10,11] The in vitro potencies of PGAs for the FP receptor have been tested in binding assays and functional assays in which FP receptors mediate various functional responses. In vitro FP receptor agonist activities of travoprost and bimatoprost have been previously shown to be greater than that of latanoprost.[12] Meta-analyses of randomized controlled clinical trials comparing the IOP-lowering effects of PGAs led both Li et al.[13] and Cheng et al.[14] to conclude that latanoprost, travoprost, and bimatoprost had equivalent efficacies. Orme et al.[15] concluded that the efficacy of latanoprost and bimatoprost were equivalent, but greater than that of travoprost. In monkey studies, there were no significant differences in IOP-lowering effects among latanoprost, travoprost, and bimatoprost.[16] The potency of PGAs for the FP receptor was, thus, thought to be sufficient to demonstrate maximum pharmacologic action (i.e., IOP-lowering effects of PGAs are apparently not enhanced through an enhancement of FP receptor agonist activity).

Among the therapeutic potential of other prostanoid receptors, a novel mechanism of action for PGAs via the prostanoid EP3 receptor has been recently reported.[5] The EP3 receptor is primarily expressed in trabecular meshwork cells; endothelial cells lining Schlemm's canal; and collector channels, aqueous veins, ciliary epithelium, ciliary muscle, and ciliary body stromal cells.[17] The EP3 receptors have distinct cellular distributions in tissues involved in uveoscleral and conventional pathways that regulate IOP.[6] In fact, topically applied EP3 receptor agonists, such as RS-6156 and RS20216, alone have been reported to lower IOP in animals. On the other hand, sulprostone, which also has EP3 agonistic activity, is not effective in lowering IOP in monkeys.[18] The difference in efficacies of some agonists may be due to the affinity of the agonists for other prostanoid receptors. For example, sulprostone is reported as having affinity for both EP1 and EP3 receptors,[19] whereas RS-61565 and RS-20216 have agonistic activity against the EP3, FP, and TP receptors.[20] Therefore, these IOP-lowering effects may have been interfered with by agonistic activities other than EP3 receptors.

The difference may be attributed to the isoform of the target EP3 receptor. The EP3 receptor isoforms are reported to exist as three types in mice (α to γ) and as four types in humans (I to IV).[21–23] These EP3 receptor isoforms have the same amino acid sequence in the extracellular ligand-binding domain, but have different amino acid sequences in the C-terminal (intracellular) domain, and are coupled with different G proteins. These isoforms are also reported to show similar ligand-binding properties but with different signal transduction properties.[24] Therefore, the binding affinity of compounds to EP3 receptors is considered to be similar for all isoforms; however, the response to agonists, such as IOP-lowering effect, may be different for each isoform.

The carboxylic acid of ONO-9054, ONO-AG-367, had highly specific agonist activity for EP3 and FP receptors. It showed agonistic activity at least for the $EP3_I$ isoform in humans, whereas other isoforms (II to IV) have not been investigated. Although reports have not been published regarding which EP3 receptor isoforms are related to IOP in humans and animals, strong agonists for the $EP3_I$ isoform may be associated with IOP responses. To clarify the mechanism of IOP regulation via the EP3 receptor and the role of its isoform, an evaluation of the expression of EP3 receptor isoforms and the effects of ONO-9054 on aqueous humor dynamics is necessary.

In conclusion, we have shown that ONO-9054 was able to reduce IOP more strongly and sustainably than PGAs via the stimulation for both EP3 and FP receptors. Thus, ONO-9054 may be useful for the treatment for glaucoma.

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Disclosure: **S. Yamane**, Ono Pharmaceutical Co., Ltd. (E); **T. Karakawa**, Ono Pharmaceutical Co., Ltd. (E); **S. Nakayama**, Ono Pharmaceutical Co., Ltd. (E); **K. Nagai**, Ono Pharmaceutical Co., Ltd. (E); **K. Moriyuki**, Ono Pharmaceutical Co., Ltd. (E); **S. Neki**, Ono Pharmaceutical Co., Ltd. (E); **F. Suto**, Ono Pharmaceutical Co., Ltd. (E); **T. Kambe**, Ono Pharmaceutical Co., Ltd. (E); **Y. Hirota**, Kazuhito Kawabata, Ono Pharmaceutical Co., Ltd. (E)

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