Pharmacologic Characterization of Omidenepag Isopropyl, a Novel Selective EP2 Receptor Agonist, as an Ocular Hypotensive Agent

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PURPOSE. The objective of this study was to investigate the pharmacologic characteristics of omidenepag isopropyl (OMDI), a compound developed as a novel intraocular pressure (IOP)-lowering agent, with better IOP control and fewer side effects than other prostanoid receptor agonists such as prostaglandin F receptor (FP) agonists.

METHODS. Binding activities of OMDI and its hydrolyzed form, omidenepag (OMD), to human recombinant prostanoid receptors (DP1-2, EP1-4, FP, and IP) were evaluated. Based on these binding assays, the agonistic activities of OMDI and OMD were further evaluated using cultured cells expressing selected prostanoid receptors. The pharmacokinetics of OMDI after topical administration was assessed in rabbits by measurement of the concentrations of both OMDI and OMD in aqueous humor. The ocular hypotensive effect of OMDI was evaluated in ocular normotensive rabbits, dogs, and both ocular normotensive and hypertensive monkeys.

RESULTS. OMDI was determined to be a selective EP2 receptor agonist. OMDI weakly bound to EP1; however, the agonistic activity of OMDI to this receptor was not demonstrated in the functional assay. After topical administration of OMDI, OMD was detected in aqueous humor whereas OMDI was not detectable. OMDI significantly lowered IOP in both ocular normotensive and hypertensive animals. The significant ocular hypotensive effects of OMDI were demonstrated by both single and repeated dosing, and its effective duration suggests sufficient efficacy by once-daily dosing.

CONCLUSIONS. These studies demonstrated that OMDI is hydrolyzed in the eye to OMD, an EP2 receptor agonist, with a significant ocular hypotensive effect in both ocular normotensive and hypertensive animal models.

Keywords: intraocular pressure, EP2 receptor agonist, omidenepag isopropyl, ocular normotensive animals, ocular hypertensive monkey

Glaucoma is a major ocular disorder that can result in blindness through an irreversible loss of visual function.1,2 Decreasing intraocular pressure (IOP) is currently the only proven treatment to prevent glaucomatous visual field loss.3–5 Currently, available IOP-lowering agents for glaucoma treatment are categorized into five main classes: β-adrenergic blocker, carbonic anhydrase inhibitor, prostaglandin F receptor agonist (FP agonist), 9-2 adrenergic agonist, and cholinergic.7,8 The launch of FP agonists such as latanoprost, travoprost, and tafluprost has led to improved glaucoma treatment on the basis of their preferable drug profiles as IOP-lowering agents, strong efficacy, tolerable ocular safety, lack of severe systemic side effects,9 and once-daily dosing leading to improved compliance. However, given the necessity of adjunctive treatment in approximately 30% of glaucoma patients10,11 and local adverse reactions associated with FP agonists affecting compliance,9,12 there remains a need for novel pharmacotherapies. Next-generation drugs for treating glaucoma would be expected to deliver stronger and/or longer-lasting ocular hypotensive efficacy, with better safety (i.e., an improved therapeutic window) and the potential for combination with existing drugs.13 Prostaglandin E2 (PGE2), as well as other EP2 receptor agonists (EP2 agonists), have been shown to reduce IOP when applied topically to human and animal eyes.14–18 PGE2, an endogenous ligand for all EP receptor subtypes (EP1, EP2, EP3, and EP419), is present in aqueous fluid20 and, in addition to modulating IOP, induces responses throughout the body such as contraction and relaxation of smooth muscle, secretion of neurotransmitters, and regulation of cell proliferation.21 EP2 is a G-protein–coupled receptor, and its wide expression in ocular tissues has been confirmed, including in cornea, conjunctiva, sclera, trabecular meshwork (TM), lens, iris, ciliary body, choroid, and retina.22 TM and ciliary muscle (CM), a part of the ciliary body, comprise the conventional and uveoscleral outflow routes, respectively.23,24 Nilsson et al.15 reported that
butaprost, a selective EP2 agonist, briefly increased uveoscleral outflow and also induced morphologic changes in the conventional outflow pathway after long-term treatment. On the basis of this documented expression of EP2 receptor in ocular tissues, and from previous results regarding aqueous dynamics, IOP reduction by EP2 agonists can be predicted to be a result of enhanced outflow via both uveoscleral and conventional routes. Therefore, EP2 agonists have the potential to be novel IOP-lowering agents, in part owing to mechanisms of action different from existing glaucoma drugs.

The purpose of this study was to assess the pharmacologic/pharmacokinetic characteristics of a novel EP2 agonist, omidenepag isopropyl (OMD; development code: DE-117), as a prospective ocular hypotensive agent. To this end, we first evaluated the in vitro receptor binding profile, comparing the agonistic activities of OMDI and its hydrolyzed form omidenepag (OMD) toward different prostanoid receptors, and then compared these two compounds with respect to corneal penetration after topical administration in rabbits. Having found that OMDI has more pronounced penetration into the aqueous compartment than OMD when formulated as an eye drop, we then assessed the IOP-lowering efficacy of topically applied OMDI in several animal models.

**Materials and Methods**

**Chemicals and Drug Preparation**

OMDI and OMD (Fig. 1) were synthesized by Ube Industries Ltd. (Yamaguchi, Japan). An ophthalmic solution for these compounds was formulated using borate buffer containing polyoxyl 35 castor oil, EDTA (disodium salt), glycerin, and benzalkonium chloride with sorbic acid as a preservative. Xalatan (0.005% [wt/vol] latanoprost ophthalmic solution) was purchased from Pfizer Japan, Inc. (Tokyo, Japan).

**Receptor Binding Assays**

All binding studies for prostanoid receptors DP1, EP1-4, FP, and IP were performed by Eurofins Cerep (Celle-Lévescault, France), while that for DP2 was carried out by Sekisui Medical Co., Ltd. (Ibaraki, Japan); binding studies were executed according to the standard assay protocols of each facility. Briefly, assays employed combining recombinant receptors, labeled tracers, and nonspecific compounds with or without test substances, and with incubation conditions as shown in Table 1. To obtain the recombinant receptors, the membrane fraction was prepared from cells that forcibly expressed each receptor as shown in Table 1 and used for the binding assay. OMDI was tested in all assays at 0.1, 0.3, 1, 3, and 10 μM. OMD was tested with respect to candidate receptors as follows: for DP1, DP2, EP1, EP3, and EP4, at 0.1, 0.3, 1, 3, and 10 μM; for EP2, at 50 pM and 0.1, 0.3, 1, 3, 10, 50, and 100 nM; and for FP and IP, at 1, 10, and 100 nM and 1 and 10 μM. Ki values of the test substances were calculated using the Cheng-Prusoff equation. Three experiments were independently performed with duplicate measurements for each sample, and mean Ki was calculated from these triplicate values.

**Functional Assays**

Functional assays, with selection of receptors based on the results of the previous binding assays, were performed by Eurofins Cerep. OMDI was evaluated with respect to its agonist activity toward EP1, EP2, and FP receptors, and OMD was assayed for the EP2 receptor only. Experimental procedures were performed according to the standard assay protocol of Eurofins Cerep. Assay conditions such as source of recombinant receptor, stimulus, incubation time, and temperature were varied, as depicted in Table 2. For agonism of EP1 and FP receptors, the cells were first treated with Fluo 4 fluorescent dye, and then the test compounds were added. Subsequently, the changes in intracellular [Ca2+] were detected as the changes in fluorescence intensity. For agonism of EP2 receptor, the changes in intracellular cAMP concentration were measured using a homogeneous time-resolved fluorescence (HTRF) assay after treatment with the test compounds in the cells. OMDI was tested in EP1, EP2, and FP assays at 1, 10, and 100 nM and 1 and 10 μM. OMD was tested in the EP2 assay at 1, 10, and 100 pM, 1, 10, and 100 nM, and 1 and 10 μM. The EC50 values were determined by nonlinear regression analysis of the concentration-response curves generated with mean replicate values using Hill equation curve fitting. Three experiments were independently performed with duplicate measurements for each sample, and mean EC50 was calculated from these triplicate values.

**Table 1. Conditions of Receptor Binding Assays**

<table>
<thead>
<tr>
<th>Recombinant Receptor, Cells</th>
<th>Labeled Tracer</th>
<th>Nonspecific Compound</th>
<th>Incubation</th>
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<tbody>
<tr>
<td>DP1 Human recombinant, 1321N1 cells</td>
<td>1.5 nM [3H] PGD2</td>
<td>1 μM GW245C</td>
<td>Time 60 min, Temperature RT</td>
</tr>
<tr>
<td>DP2 Human recombinant, CHEM-1 cells</td>
<td>1.6–1.9 nM [3H] PGD2</td>
<td>10 μM PGD2</td>
<td>60 min, 25°C</td>
</tr>
<tr>
<td>EP1 Human recombinant, HEK-293 cells</td>
<td>1.5 nM [3H] PGE2</td>
<td>10 μM PGE2</td>
<td>120 min, RT</td>
</tr>
<tr>
<td>EP2 Human recombinant, HEK-293 cells</td>
<td>3 nM [3H] PGE2</td>
<td>10 μM PGE2</td>
<td>120 min, RT</td>
</tr>
<tr>
<td>EP3 Human recombinant, HEK-293 cells</td>
<td>0.5 nM [3H] PGE2</td>
<td>1 μM PGE2</td>
<td>120 min, RT</td>
</tr>
<tr>
<td>EP4 Human recombinant, HEK-293 cells</td>
<td>0.5 nM [3H] PGE2</td>
<td>10 μM PGE2</td>
<td>120 min, RT</td>
</tr>
<tr>
<td>FP Human recombinant, HEK-293 cells</td>
<td>2 nM [3H] PGF2α</td>
<td>10 μM cloprostenol</td>
<td>60 min, RT</td>
</tr>
<tr>
<td>IP Human recombinant, HEK-293 cells</td>
<td>6 nM [3H] iloprost</td>
<td>10 μM iloprost</td>
<td>60 min, RT</td>
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RT, room temperature.

**Figure 1.** Chemical structures of omidenepag isopropyl (OMDI) and omidenepag (OMD). (a) Omidenepag isopropyl. (b) Omidenepag.
Omidenepag Isopropyl as a Novel IOP-Lowering Agent

### Table 2. Conditions of Functional Assays

<table>
<thead>
<tr>
<th>Source, Cells</th>
<th>Stimulus, Control</th>
<th>Incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>EP1 Human recombinant, HEK-293 cells</td>
<td>None, 100 nM PGE$_2$</td>
<td>Not specific time, RT</td>
</tr>
<tr>
<td>EP2 Human recombinant, CHO-K1 cells</td>
<td>None, 10 µM PGE$_2$</td>
<td>30 min, 5°C</td>
</tr>
<tr>
<td>FP Human recombinant, HEK-293 cells</td>
<td>None, 1 µM PGE$_2$</td>
<td>Not specific time, RT</td>
</tr>
</tbody>
</table>

RT, room temperature.

**Animals**

Japanese white rabbits (Kitayama Labes, Co., Ltd., Nagano, Japan) were used for pharmacokinetic and IOP studies. Beagle dogs (Kitayama Labes) and cynomolgus monkeys (KEARI Co., Ltd., Osaka, Japan; Shin Nippon Biomedical Laboratories, Ltd., Tokyo, Japan) were also used in IOP studies. Ocular hypertension in monkeys was induced by laser irradiation of the TM as published previously.$^{2,3}$ All experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the internal ethics code for animal study of Santen Pharmaceutical Co., Ltd. (Nara, Japan). Animals were housed under a 12-hour light–dark cycle with specific on/off times of light for each animal.

**Pharmacokinetic Study**

Aqueous humor was collected 1, 2, and 4 hours after topical administration of test solutions of OMDI at 0.1% or OMD at 1%. The collected aqueous humor samples were pretreated by a solid-phase extraction method, and injected for liquid chromatography–tandem mass spectrometry (LC-MS/MS) to determine the concentrations of OMDI and OMD.

**IOP Study**

A calibrated pneumotonometer (Model 30 Classic; Reichert, Depew, NY, USA) was used to measure IOP in rabbits and monkeys, and tonometry using the Icare TonoVet Tonometer (Icare Finland Oy, Vantaa, Finland) was done in dogs. IOP measurements were performed on conscious animals, and included (with the exception of dogs) local anesthesia induced by topical administration of 0.4% oxybuprocaine hydrochloride solution (Benoxil ophthalmic solution 0.4%, Santen Pharmaceutical Co., Ltd.). IOP was measured at $t = 0$ for baseline and at the following time points after administration of compounds: 2, 4, and 6 hours in rabbits, dogs, and ocular hypertensive monkeys; 2, 4, 6, and 8 hours in ocular normotensive monkeys.

All test solutions and vehicles were administered unilaterally, with the fellow eye remaining untreated except in dogs. In dogs, right eyes were treated with drugs, with vehicle being administered to contralateral eyes. Administration volume was 50 µL in rabbits and dogs and 20 µL in monkeys. The tested concentrations of OMDI were determined based on the preliminary in vitro and in vivo studies to suit each study purpose as follows: 0.001%, 0.01%, and 0.05% in rabbits; 0.0006% in dogs; 0.0001%, 0.001%, and 0.01% in ocular normotensive monkeys; 0.01% in ocular hypertensive monkeys. Administration was performed at approximately 1 PM (Zeitgeber time [ZT] 11) in rabbits or 10 AM (ZT3) in other experiments. Drug-induced IOP changes from baseline IOP were compared with IOP changes in the vehicle-treated group.

Dosing frequency was as follows: single, in rabbits and ocular hypertensive monkeys; once a day for 28 days, in dogs; once a day for 7 days, in ocular normotensive monkeys. Masked observers measured IOP in all experiments.

**Ocular Toxicity Study**

For evaluation of ophthalmic toxicity induced by administration of OMDI, eyes of monkeys were evaluated by gross examination, slit-lamp, funduscopic, and histopathologic examination. Additionally, changes in central corneal thickness (CCT), IOP, corneal endothelium, and retinal function were evaluated by pachymetry, pneumotonometer, specular microscopy, and ERG, respectively. All examinations except gross observation were performed on subjects that were under systemic anesthesia induced by intramuscular injection of ketamine hydrochloride (Kamud Drugs Pvt. Ltd., Maharashtra, India). Examination was performed 4 weeks and 13 weeks after the start of dosing except IOP measurement (3 weeks and 12 weeks).

Test solutions containing 0.003%, 0.01%, or 0.03% OMDI, as well as vehicle, were administered unilaterally once a day for 13 weeks with the fellow eye remaining untreated. Drug-induced changes in evaluation index from baseline were compared with changes in the vehicle-treated group. All groups consisted of 6 animals (3 males and 3 females) except the 0.03% OMDI-treated group, which had 10 animals including 4 animals (2 males and 2 females) specifically assigned to assess the reversibility of toxicity during a 4-week recovery period.

**Data Analysis**

In the IOP studies using rabbits and ocular normotensive monkeys and the ocular toxicity study, data were analyzed by a sequence of Bartlett and Dunnnett’s test or using Steel’s post hoc test, comparing the vehicle- and OMDI-treated groups. OMDI data in the IOP study using ocular hypertensive monkeys and data for latanoprost in both monkey models versus the vehicle-treated group were analyzed by $F$-test first for equality of variance, followed by Student’s $t$-test or Aspin–Welch’s $t$-test. Data in the IOP study using dogs were analyzed by paired $t$-test. $P < 0.05$ was considered to indicate statistical significance.

**Results**

**Binding Affinities of OMDI and OMD to Prostanoid Receptors**

OMDI weakly bound to the EP1 receptor with $K_i = 1700$ nM, to the EP2 receptor with $K_i > 4600$ nM, to the FP receptor with $K_i > 4500$ nM (Table 3). $K_i$ values for OMDI binding to DP1-2, EP3, EP4, and IP receptors could not be calculated because OMDI did not yield any measurable binding data to these receptors at the evaluated concentrations (Table 3). On the other hand, OMD bound to the EP2 receptor strongly with a $K_i$ value of 3.6 nM and showed no binding affinity to other
receptors (Table 3). The lack of measurable binding to the FP receptor may be interpreted as demonstrating that OMD has a totally different pharmacologic mode of action for IOP reduction from that for FP agonists in current clinical use, such as latanoprost, travoprost, and tafluprost. These compounds have strong binding affinity to FP receptor but very weak or no binding affinity to EP2 receptor.24–26 In our hands, these FP agonists also showed selective binding to the FP receptor, but no binding affinity to the EP2 receptor.

**Agonistic Activities of OMDI and OMD to Prostanoid Receptors**

OMDI showed only weak affinity for EP1, EP2, and FP receptors in the binding assays (Table 3), however, its EC50 for all three receptors was above 10,000 nM, the highest concentration of OMDI tested in each assay. On the other hand, OMD was tested for agonism versus the EP2 receptor, showing strong activity with an EC50 of 8.3 nM (Table 4), indicative of a selective EP2 receptor agonist.

**Pharmacokinetic Profile of OMDI and OMD**

The acid forms of FP receptor agonists have been reported to show poor corneal penetration compared to their ester forms27,28; therefore, we compared the penetration of OMDI and OMD into the anterior chamber in rabbits to select the optimal chemical structure for development.

OMD was the only detectable form in the aqueous humor after the topical administration of either OMDI or OMD (Fig. 2), as any signal(s) for OMDI following administration was below the lower limit of quantification (<1 ng/mL). The maximum concentrations (Cmax) of OMD measured in aqueous humor after administration of OMDI and OMD were 108.0 ± 23.0 and 11.4 ± 10.7 ng/mL (mean ± SD), respectively. Although the administration dosage of OMDI was 1/10 of OMD, the OMD Cmax corresponding to OMDI instillation was clearly higher than that of OMD itself, and was attained at 2 hours after administration (Fig. 2).

**IOP-Lowering Effect of OMDI in Ocular Normotensive Animals**

Based on the previous pharmacokinetic study (Fig. 2), we selected OMDI, as opposed to OMD, as a candidate development compound for an ophthalmic solution product. After OMDI administration in rabbits, which took place during the late period of the light phase (ZT11), IOP changes were evaluated during the dark phase of the light cycle (Fig. 3). The predosing value of IOP (mean ± SE) ranged from 18.7 ± 0.8 to 18.8 ± 1.2 mm Hg, with no significant differences between the groups. After drug administration, IOP increased over the next 2 hours in all groups toward levels routinely observed for the dark phase (Fig. 3), reflecting the circadian change in IOP normally seen in ocular normotensive rabbits.29 Following this expected increase in IOP, OMD decreased over the next 4 hours in a statistically significant fashion in OMDI-treated groups compared to the vehicle-treated group (Fig. 3). Six hours after initial administration, at which time the maximal effect was detected among measurement points, the mean IOP reductions compared to vehicle were 6.4, 9.0, and 8.8 mm Hg, at doses of 0.001%, 0.01%, and 0.03% of OMDI, respectively (Fig. 3). The weaker efficacy of 0.001% OMDI compared to 0.01% and 0.03% suggested a dose-dependent effect of OMDI on rabbit IOP, but

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<tbody>
<tr>
<td>OMDI</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>1,700</td>
<td>&gt;4,600</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
</tr>
<tr>
<td>OMD</td>
<td>&gt;6,800</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>3.6</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
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K<sub>i</sub> value of the test substances was calculated using the Cheng-Prusoff equation. Data represent mean of three values calculated from independent experiments with duplicate determinations.

For IOP changes, we calculated the EC<sub>50</sub> values of the test substances using nonlinear regression analysis of the concentration–response curves generated with mean replicate values using Hill equation curve fitting. Data represent mean of three values calculated from independent experiments with duplicate determinations.

**Figure 2.** Aqueous concentrations of OMD in rabbits. Aqueous humor in rabbits was collected 1, 2, and 4 hours after topical administration of either 0.1% OMDI or 1% OMD. OMD concentrations were determined using LC-MS/MS. Data represent the mean ± SD of 4 eyes.
this was difficult to discern, given the similar efficacy between 0.01% and 0.03% at both the 4- and 6-hour time points.

IOP changes in dogs and ocular normotensive monkeys were evaluated during the light phase. Predosing values of IOP (mean ± SE) were 15.6 ± 0.5 and 15.5 ± 0.3 mm Hg (drug- and vehicle-treated eyes in dogs, respectively) or ranged from 16.6 ± 1.2 to 17.9 ± 0.6 mm Hg (monkeys) with no significant differences between the groups. In OMDI-treated dogs, IOP decreased significantly for almost all measurement points (Fig. 4). Over a 4-week period, reductions in IOP from its predosing value on day 1 ranged from approximately 4 to 6 mm Hg by 6 hours after administration, when the maximal reduction for all evaluated time points was reached in all groups (Fig. 4). The significant and sustained decrease in IOP following drug administration (indicated by arrows following time = 0) for days 7, 14, and 28 (Fig. 4) suggested that once-a-day dosing was suitable for maintaining the efficacy duration of OMDI.

OMDI also showed significant and dose-dependent IOP-lowering effects at doses of 0.0001%, 0.001%, and 0.01% in ocular normotensive monkeys (Fig. 5a), with mean maximal IOP reductions of 2.4 ± 0.6, 7.6 ± 1.7, and 13.3 ± 1.2 mm Hg at each tested concentration, respectively (Fig. 5b). The significant decreases in IOP for 0.001% and 0.01% OMDI at time 0 of day 7 (Fig. 5a) suggested that its efficacy lasted more than 24 hours, emulating the result in dogs. In some eyes receiving the two higher concentrations (one eye at 0.01% and six eyes with 0.01%), the IOP actually decreased to below 5 mm Hg, a level representing hypotony, as a real measurement value. Latanoprost was evaluated as a positive control compound, and we confirmed its significant and consistent effect on IOP at a clinically relevant dose, commensurate with our previous reports, although the mean maximum IOP reduction for Xalatan was only 33% of that for 0.001% OMDI (Fig. 5b).

**IOP-Lowering Effect in Ocular Hypertensive Animals**

Since OMDI showed significant and strong effects on IOP in normotensive animals by topical administration (Figs. 3–5), we next evaluated its efficacy in laser-induced ocular hypertensive monkeys. The predosing value of IOP (mean ± SE) ranged from 34.6 ± 2.3 to 36.2 ± 2.7 mm Hg with no significant differences. OMDI at 0.01% significantly decreased IOP at all measurement points following administration (Fig. 6a), and the maximal reduction achieved through the 6-hour evaluation period was 19.9 ± 3.0 mm Hg (Fig. 6b). Latanoprost also showed significant IOP-lowering effect with maximal reduction of 9.7 ± 1.8 mm Hg (Figs. 6a, 6b), again, similar to what was found previously.

**Ocular Toxicity**

During the 13-week dosing period and 4-week recovery period, no drug-related changes were observed in monkey eyes, either by gross, slit-lump, funduscopic, or histopathologic examinations, or by specular microscopy or ERG; notably,
FIGURE 5. Changes in IOP after administration of OMDI in ocular normotensive monkeys. OMDI at 0.0001%, 0.001%, or 0.01%, Xalatan, or vehicle was topically administered to one eye in ocular normotensive monkeys. IOP change after drug administration was compared to the predosing baseline value established on day 1. Data represent mean ± SE for 8 eyes. Arrows indicate the timing of administration. *P < 0.05, **P < 0.01 relative to vehicle-treated group (Student’s t-test). †††P < 0.001 relative to vehicle-treated group (Aspin-Welch’s t-test). ‡‡‡P < 0.01 relative to vehicle-treated group (Steel’s test). (a) IOP changes from baseline at measurement time point, (b) maximal IOP reduction following topical application of OMDI.
among the animals used for this study have the closest genetic relationship, as well as the most congruent anterior angle anatomy, compared to humans (Figs. 5, 6). The IOP of normotensive monkeys treated with OMDI at concentrations above 0.001% was decreased in some cases to below 5 mm Hg, the minimum level of IOP limited by the existence of episcleral venous pressure. Such a strong effect on IOP of OMDI, however, did not affect retinal function as measured by ERG and did not cause any histopathologic changes in ocular tissues. Nevertheless, this phenomenon should be carefully monitored in clinical studies.

It has been proposed that the IOP-lowering effect of EP2 agonists is induced by enhancement of both conventional and uveoscleral outflow routes.\textsuperscript{15} Butaprost\textsuperscript{16} and PF-04217329, an EP2 agonist, which like OMDI has a non-prostaglandin structure with an IOP-lowering effect in animals,\textsuperscript{15} and were shown to lower IOP by increasing uveoscleral outflow in animal models. Butaprost and another EP2 agonist, AH13205, cause relaxation of CM, which constitutes a part of the uveoscleral outflow apparatus,\textsuperscript{31,32} and therefore the enhancement of drainage via this route by EP2 receptor agonists can be assumed to be due to an increase in the CM intermuscular space as a result of its relaxation. Indeed, Richter et al.\textsuperscript{33} reported that AH13205 caused enlargement of spaces between CM bundles and suggested that it was caused by remodeling of the extracellular matrix. In addition to the enhancement of uveoscleral outflow, EP2 receptor agonists may facilitate conventional outflow (via TM and Schlemm’s canal), since Nilsson et al.\textsuperscript{15} reported that repeated dosing of butaprost led to increased intercellular spaces and decreased collagenous materials in these tissues. Enhancement of both outflow routes may contribute to the strong effect of OMDI on IOP. Additionally, a simultaneous effect on both routes may be indicative of a mode of action being different from other IOP-lowering agents such as FP agonists, suggesting that an adjunctive effect with other agents also could be expected in clinical practice.

Despite its strong effect on IOP, OMDI did not show significant or severe ocular adverse effects such as corneal edema or conjunctival redness in both the IOP studies and the ocular toxicity evaluations. The high selectivity of OMD for the EP2 receptor, and lack of specific functional effects of OMDI with respect to other receptors, would be expected to contribute to the observed good tolerability (Tables 3, 4).

Selectivity of OMD for the EP2 receptor has also been confirmed by a panel assay encompassing a wide variety of receptors (data not shown). This selectivity of OMD for the EP2 receptor could be due to its non-prostaglandin structure (Fig. 1), in contrast to PGE\textsubscript{2}, which indeed is a prostanoid and shows binding affinities to all EP receptor subtypes.\textsuperscript{19} Limiting the EP subtype specificity of an IOP-lowering agent would be therapeutically sound, since stimulation of the EP3 receptor has an opposite effect on intracellular cAMP compared to EP2 and EP4 receptors, as EP3 decreases cAMP while EP2 and EP4 both increase cAMP.

OMDI slightly increased CCT. The change was not statistically significant when compared with the vehicle-treated group, for both sexes, and more importantly, it was reversible. PF-04217329, an EP2 agonist, which like OMDI has a non-prostaglandin structure with an IOP-lowering effect in animals and human patients,\textsuperscript{16,18} has a similar effect on CCT as well. CCT has been reported to change even without drug administration in circadian fashion.\textsuperscript{34,35} Actually, changes in CCT are common among many existing IOP-lowering drugs. For example, FP agonists decrease CCT while β-blockers\textsuperscript{36} and α-2 agonists\textsuperscript{37} increase CCT. It is unclear what caused the change in CCT with OMDI treatment, and this side effect would be expected to be monitored in any clinical setting.

In summary, OMDI is converted to the active product OMD during corneal penetration, and OMD is a highly selective EP2 agonist; and (3) this hydrolyzed form exhibits significant IOP-lowering activity in several animal models, under both ocular normotensive and hypertensive conditions (Figs. 2–6).

We have shown that (1) OMD, a pharmacologically active form of OMDI, is a selective EP2 agonist based on both binding and functional assays (Tables 3, 4); (2) following topical administration, OMDI is converted to OMD during corneal penetration; and (3) this hydrolyzed form exhibits significant IOP-lowering activity in several animal models, under both ocular normotensive and hypertensive conditions (Figs. 2–6). OMDI demonstrated strong IOP-lowering effects in monkeys, which among the animals used for this study have the closest genetic
receptor agonist. Topically administered OMDI significantly lowered IOP in several animal models, including ocular hypertensive monkeys, without significant adverse effects. Although further investigations are needed to clarify the effects of OMDI on aqueous humor dynamics and to understand the mechanism underlying its IOP-lowering effect, OMDI shows promise as a novel ocular hypotensive agent that could be adjunctively used in combination therapy with existing IOP-lowering agents, resulting in IOP-lowering efficacy in non-responders or low responders.

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