Effects of a Novel Selective EP2 Receptor Agonist, Omidenepag Isopropyl, on Aqueous Humor Dynamics in Laser-Induced Ocular Hypertensive Monkeys

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Abstract

Purpose: To investigate the mechanism of the intraocular pressure (IOP)-lowering effect of a novel selective prostaglandin E2 receptor 2 (EP2) receptor agonist, omidenepag isopropyl (OMDI).
Methods: The effect of OMDI on IOP and aqueous humor dynamics was evaluated in cynomolgus monkeys with unilateral laser-induced ocular hypertension. In a crossover manner, the hypertensive eye of each monkey was dosed once daily with $20\,\mu L$ of either 0.002% OMDI or vehicle. On day 7 of dosing, IOP was measured by pneumatonometry, aqueous humor flow and outflow facility were evaluated by fluorophotometry, and uveoscleral outflow was calculated mathematically. Treatments were compared by paired *t*-tests.
Results: OMDI at 0.002% significantly lowered IOP by 27%, 35%, and 44% at 0.5, 1.5, and 4 h after the last dosing, respectively. There was no difference in aqueous humor flow between vehicle and OMDI treatments. When comparing OMDI to the vehicle treatment, outflow facility and uveoscleral outflow were significantly ($P < 0.05$) increased by 71% and 176%, respectively.
Conclusions: OMDI, a novel IOP-lowering compound, reduced IOP by increasing outflow facility and uveoscleral outflow in nonhuman primates.

Keywords: glaucoma, aqueous humor dynamics, EP2 receptor, intraocular pressure

Introduction

GLAUCOMA, A NEURODEGENERATIVE disease characterized by selective loss of retinal ganglion cells followed by visual field defects, is the leading cause of irreversible blindness worldwide.[1–4] Elevated intraocular pressure (IOP) is a major factor for glaucoma, and IOP reduction remains the main goal of current glaucoma therapy.[3,5–7] Glaucoma patients are treated initially with pharmacological monotherapy, such as prostaglandin F (FP) receptor agonists (FP agonists) or beta-adrenergic receptor blockers (beta blockers). However, ~30% of glaucoma patients require adjunctive therapy within 1 year, and >40% of glaucoma patients rely on adjunctive

therapy to control their IOP; hence, 2 or more drugs are often added to the treatment regimen.[8–12] From a safety viewpoint, beta blockers may cause serious systemic side effects in the cardiovascular and respiratory systems,[13–15] and FP agonists can stimulate pigmentation in periocular skin and iris, deepen the upper eyelid sulcus, and cause abnormal elongation of eyelashes.[16–18] As such, there remains a need for more potent and safer IOP-lowering drugs with novel mechanisms of action.

The prostaglandin E2 receptor 2 (EP2) receptor could be an alternative target for IOP regulation. It is a G-protein coupled receptor found in several ocular tissues, including nonpigmented ciliary epithelium, trabecular meshwork (TM),

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and ciliary muscle (CM),[19,20] tissues that are associated with aqueous humor dynamics (AHD). Prostaglandin E_2 (PGE$_2$) is an endogenous ligand for EP receptors; these receptors have 4 subtypes as follows: EP1, EP2, EP3, and EP4.[21,22] Although PGE$_2$ has a potent IOP-lowering effect, it is associated with some adverse effects.[23] Several promising agonists to EP2 receptor have shown IOP-lowering effects with fewer adverse effects than those of PGE$_2$.[24–26] Omidenepag isopropyl (OMDI) is one such compound.[27] After topical instillation, OMDI is converted to omidenepag (OMD) during corneal penetration.[27] OMD is a highly selective EP2 receptor agonist ($K_i = 3.6$ nM in binding affinity, and EC$_{50} = 8.3$ nM in agonistic activity) with a non-prostaglandin structure (Fig. 1).[27] The IOP-lowering effect of OMDI has been demonstrated in several animal models, including rabbits, dogs, and monkeys, and in patients with primary open-angle glaucoma or ocular hypertension,[27,28] which suggests that OMDI has potential for future glaucoma treatment. The current study tests the hypothesis that OMDI lowers IOP by improving outflow through both aqueous humor drainage pathways, a mechanism somewhat different from other EP2 agonists.[29,30]

In this study, we investigated the mechanism of the IOP-lowering effect of OMDI by examining AHD in ocular hypertensive monkeys.

Methods

Drugs

OMDI was synthesized by Ube Industries, Ltd. (Yamaguchi, Japan) (Fig. 1). An ophthalmic solution of 0.002% OMDI and its vehicle were formulated by Santen Pharmaceutical Co., Ltd. (Osaka, Japan). The vehicle contained citrate buffer, polyoxyl 35 castor oil, edetate disodium, glycerin, pH adjuster, water, and benzalkonium chloride as a preservative. Acetazolamide was purchased from Ben Venue Laboratories, Inc. (Bedford, OH).

Animals

Fifteen female cynomolgus monkeys (Charles River Laboratories, Inc., Wilmington, MA or Covance, Inc., Princeton, NJ) had laser treatments to the TM of the left eye to create unilateral laser-induced ocular hypertension.[31] The lasering had been done between 5 and 13 years before the study. Fifty to 100 argon laser photocoagulation burns, with a spot size of 50 microns, a power of 1,000 mW, and an exposure time of 0.5 s were applied to the midportion of the

FIG. 1. Chemical structures of OMDI and OMD. **(A)** OMDI, **(B)** OMD. OMDI, omidenepag isopropyl; OMD, omidenepag.

TM for 340 degrees. The animals were between 9 and 18 years of age and weighing 3.2–6.6 kg when they were used in the study. Animals were housed in a temperature and humidity controlled room with lights on for 12 h per day starting at 7:00 am. All animal care and experimental procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved and monitored by the Animal Care and Use Committee at the University of Nebraska Medical Center.

AHD study

The AHD methods were similar to those described previously.[32,33] The study was performed according to the schedule described in Fig. 2. Briefly, the study was an investigator-masked crossover design of OMDI or vehicle treatment to the hypertensive eye of each animal. The effects of OMDI on AHD were determined by comparing OMDI treatment data with vehicle treatment data and with baseline measurement of IOP (before dosing). The 2 measurement days were separated in time by at least 2 weeks to allow recovery from anesthesia and for washout of drug effects. Twenty microliters of either 0.002% OMDI ophthalmic solution or vehicle were applied between 8:00 and 9:00 am to hypertensive eyes once daily for 7 days. Dosing period and concentration of OMDI ophthalmic solution were determined on the basis of our previous animal[27] and clinical data.[28,34] On day 7, ∼4 h before measurements commenced, 10% fluorescein solution was applied to the cornea as 3 to 4 topical drops and then sedation was induced by intramuscular administration of ketamine HCl (15 mg/kg). A final dose of 0.002% OMDI ophthalmic solution or vehicle was applied topically to the eye at around 9:00 am.

The cornea was anesthetized with topical proparacaine HCl 0.5%, and IOP was measured using a pneumatonometer (Model 30; Reichert, Inc., Depew, NY). Corneal thickness and anterior chamber depth were measured by slit-lamp pachymetry, cornea diameter was measured with calipers, and cornea and anterior chamber volumes were calculated from the measured values of cornea diameter, cornea thickness, and anterior chamber depth.[35] Beginning at ∼9:30 am, the fluorescence intensities of the cornea and anterior chamber were measured using a scanning ocular fluorophotometer (Fluorotron Master; OcuMetrics, Inc., Mountain View, CA). Scans were repeated thrice at 45-min intervals for a total of 4 sets of scans. The slopes of the cornea and anterior chamber fluorescein decay curves and the anterior chamber volume were used in the determination of aqueous flow (F_a; μL/min). The numerous formulas have been reported in detail elsewhere.[36,37]

Immediately after the fourth set of scans, IOP was measured (IOP$_1$; mmHg), and acetazolamide (20 mg/kg) was given by intravenous injection. This carbonic anhydrase inhibitor was used as a tool to reduce IOP and F_a and enable calculation of outflow facility. At 1.0, 1.75, and 2.5 h later, fluorophotometric scans, F_a calculations, and IOP measurements were repeated. At each of these 3 postacetazolamide periods, fluorophotometric outflow facility (C_{fl}; μL/min/mmHg) was calculated as the ratio of the change in F_a to the change in IOP from the acetazolamide administration.[38] If there was <0.1 μL decrease in F_a or <0.5 mmHg decrease in IOP at a postacetazolamide period, C_{fl} was not calculated for that period. The reported
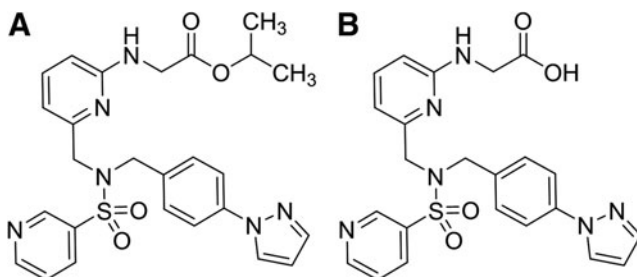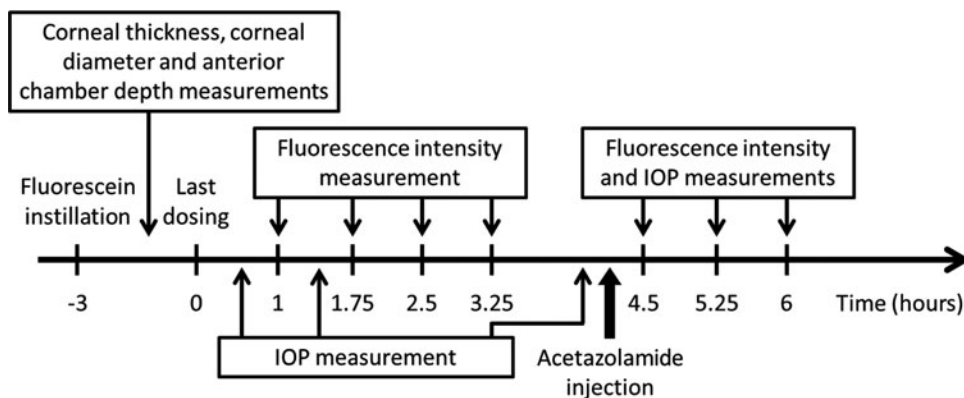
FIG. 2. Timeline of the study measurements and dosing. Fluorescein instillation and the last dosing of vehicle or 0.002% OMDI were performed at ∼6:00 and 9:00 am, respectively. IOP and fluorescence intensity were measured on the basis of the above timeline for the evaluation of AHD. AHD, aqueous humor dynamics; IOP, intraocular pressure.

C_{fl} is the average of 2 to 3 calculated values. If only one or no acceptable postacetazolamide C_{fl} value was calculated, then a mean C_{fl} was not recorded for that eye.

Episcleral venous pressure (P_{ev}; mmHg) could not be measured using the noninvasive technique of venomanometry in the monkeys because of their heavily pigmented conjunctiva that prevented visualization of the vessels. P_{ev} also could not be measured by direct cannulation because of its invasive nature. Instead, estimates were made on the basis of a prior publication[39] that used direct cannulation techniques in nonhuman primates. Based on that information, we chose the value of 14 mmHg as the estimated P_{ev} value in this study.

Uveoscleral outflow (F_{us}; μL/min) was calculated for each animal using the modified Goldmann equation, $F_{us} = F_a - C_{fl} (IOP_1 - 14)$. If any of the variables in the Goldmann equation were missing, then F_{us} could not be calculated for that eye.

IOPs and aqueous flow values were collected in 15 animals. Mean C_{fl} was reported in 8 animals due to the strict criteria for the C_{fl} calculation. Uveoscleral outflow was calculated in the same 8 animals.

Statistical analyses

IOP (mmHg), F_a (μL/min), C_{fl} (μL/min/mmHg), and F_{us} (μL/min) were analyzed. Parameters were compared between the vehicle and 0.002% OMDI-treated groups using the 2-tailed paired *t*-test. Differences were considered statistically significant if $P < 0.05$.

Results

At the beginning of the study, IOP in the laser-treated eye was found to be significantly higher than that in the fellow eye of each monkey (data not shown). No statistically significant differences in baseline IOP values were observed on days of vehicle and OMDI treatments. OMDI at 0.002% significantly lowered IOP at 0.5, 1.5, and 4 h after the last dosing by 27%, 35%, and 44% reduction compared with the vehicle treatments, respectively (Fig. 3).

When comparing 0.002% OMDI with vehicle treatments, there was no difference in aqueous humor production (Fig. 4A), an increase in outflow facility from 0.11 ± 0.03 μL/min/mmHg to 0.18 ± 0.04 μL/min/mmHg (71% increase; $P = 0.028$) (Fig. 4B), and an increase in uveoscleral outflow from 1.07 ± 0.50 μL/min to 2.94 ± 0.55 μL/min (176% increase; $P = 0.033$), respectively (Fig. 4C).

### Discussion

OMDI is a novel selective EP2 receptor agonist with a nonprostaglandin structure (Fig. 1) under development for the treatment of glaucoma as an IOP-lowering drug.[27] Based on the efficacy results of our previous animal[27] and clinical studies, we administered 0.002% OMDI for 7 days, which was expected to show a greater IOP-lowering effect compared with a single dose in monkeys. With this dosing regimen, OMDI at 0.002% significantly lowered IOP by ∼19, 23, and 26 mmHg compared to the baseline at 0.5, 1.5, and 4 h after the last dosing, respectively (Fig. 3), and increased outflow facility and uveoscleral outflow (Fig. 4). Previously, we evaluated the IOP-lowering effects of one of the FP agonists, travoprost (0.004%; TRAVATAN®), showing the reduction of IOP by ∼10 mmHg in laser-induced ocular hypertensive monkeys.[40] Recently, we also indicated that OMDI at 0.01% and latanoprost (0.005%; Xalatan®) showed the maximal IOP reductions of 19.9 and 9.7 mmHg, respectively, in laser-induced ocular hypertensive monkeys.[27] These findings suggest that at least at certain dosing regimen and measurement times, the IOP-lowering effects of OMDI are greater than those of FP agonists in laser-induced ocular hypertensive monkeys. We also reported that OMDI dose dependently lowered IOP
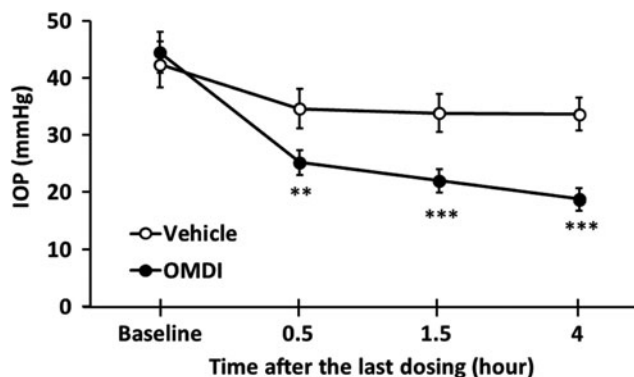
FIG. 3. Effects of OMDI ophthalmic solution on IOP in ocular hypertensive monkey eyes. IOPs were measured before the acetazolamide injection. IOPs of vehicle or 0.002% OMDI are represented as the mean ± SEM of 15 eyes. **$P < 0.01$, ***$P < 0.001$ compared with vehicle by paired *t*-test.

FIG. 4. Effects of OMDI ophthalmic solution on AHD in ocular hypertensive monkey eyes. AHD parameters following treatment with vehicle or 0.002% OMDI are represented as mean ± SEM of 15 eyes for aqueous flow **(A)** and 8 eyes for both outflow facility **(B)** and uveoscleral outflow **(C)**, respectively. *$P < 0.05$ compared with vehicle by paired t-test.
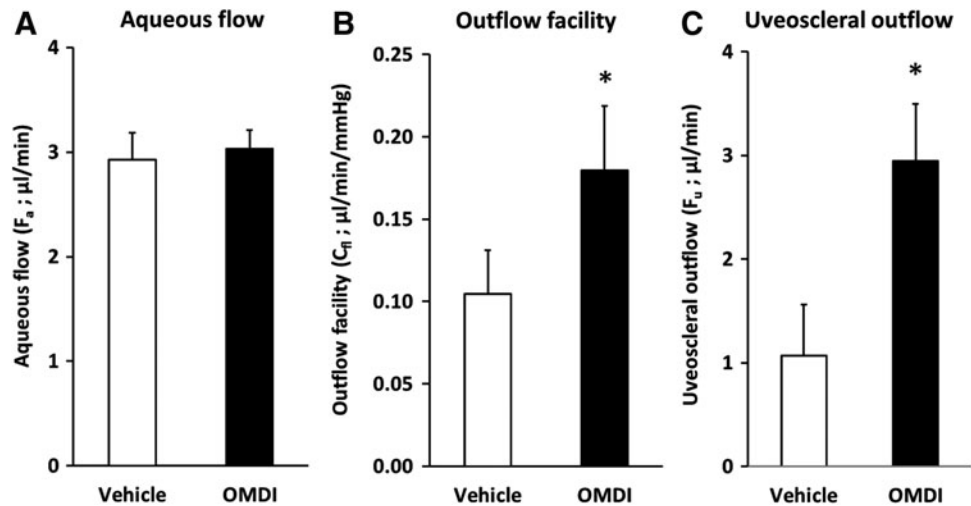
at doses of 0.0001%, 0.001%, and 0.01% in ocular normotensive monkeys with maximal IOP reductions of 2.4, 7.6, and 13.3 mmHg, respectively, and also that OMDI reached the peak of IOP-lowering effect during 4 to 8 h after the instillation.[27] In addition, the significant decreases in IOP for 0.001% and 0.01% OMDI at time 0 on day 7 of dosing (trough) suggested that its efficacy lasted more than 24 h.[27]

The outflow facility in this study was accessed by the fluorophotometric method.[35] This method directly measures changes in aqueous flow and IOP from the acetazolamide treatment. It does not apply a weight to the eye, as is done with tonography, and thus avoids the confounding factors of ocular rigidity and pseudofacility. However, it cannot separate facility of trabecular outflow from facility of uveoscleral outflow. The facility of uveoscleral outflow is small in primates[41–43]; hence, it is assumed that the changes in outflow facility measured by the fluorophotometry method mainly reflect changes in trabecular outflow facility.[44] In support of this assumption, a previous study of cats found that, when measured by direct invasive methods, uveoscleral outflow and total outflow facility were increased yet uveoscleral outflow facility was unchanged following treatment with prostaglandin A_2.[45] The conclusion could be made that prostaglandin A_2 improved outflow facility through the TM. Similarly, we could conclude with some confidence that OMDI increases trabecular outflow facility.

Our data demonstrate that OMDI has a dual mechanism of action of improving drainage through both outflow pathways. Outflow facility is low, and uveoscleral outflow rate is slow in patients with elevated IOP,[4,46] which means that OMDI targets the 2 areas of pathology in the anterior segment of glaucomatous eyes. OMDI's mechanism of action is somewhat different from other IOP lowering drugs making it potentially synergistic or additive when combined with existing IOP-lowering drugs. FP agonists lower IOP predominantly by increasing uveoscleral outflow and to a lesser extent trabecular outflow facility.[44] Beta blockers inhibit aqueous humor production.[47] The alpha 2 receptor agonist, brimonidine, also inhibits aqueous humor production but only when dosed acutely.[48] When dosed for multiple days, it increases uveoscleral outflow.[48] Rho kinase inhibitors mainly

improve outflow facility,[33,49] although an effect on P_{ev}[50] and uveoscleral outflow[33] also contribute to its IOP-lowering effect. We previously confirmed the adjunctive IOP-lowering effect of OMDI in animal models.[51] Furthermore, because of its dual mechanism of action, OMDI might be effective in patients who are less or nonresponsive to FP agonists.

EP2 receptor expression has been confirmed in the CM (part of the uveoscleral outflow pathway) and TM (part of the trabecular outflow pathway).[19] Stimulation of EP2 receptors leads to an increase in intracellular cAMP,[52] which results in relaxation of smooth muscle.[53] EP2 agonists relax both tissues and cells in the CM and TM.[54–57] Therefore, it is thought that this compound improves uveoscleral outflow and trabecular outflow facility. Nilsson et al.[30] found that butaprost, another EP2 agonist, increased uveoscleral outflow but not outflow facility. However, after dosing for a year, there was enlargement of intercellular spaces and decrease in collagen-like extracellular matrix in the TM and Schlemm's canal suggesting an improvement in outflow facility with long-term dosing. Since EP2 receptor stimulation is involved in induction of matrix metalloproteinase (MMP) protein and activation of MMPs,[58,59] the structural changes in both TM and CM tissues are probably caused by changes in the extracellular matrix. Based on these findings and our data in the current study (Fig. 4), the mechanism of the IOP-lowering effect of OMDI potentially involves increased trabecular outflow facility through expansion of the intercellular spaces, reduction of extracellular matrices and relaxation of TM, and increased uveoscleral outflow through relaxation of the CM.

Elevated IOP in glaucoma patients is caused mainly by a decrease in trabecular outflow facility.[4,46] The animal model used in this study had ocular hypertension as a result of lasering the TM that caused tissue scarring and increased resistance through the outflow pathway. This model is similar to human glaucoma in that it has reduced outflow facility, nerve fiber layer defects, and progressive enlargement of the cup-to-disc ratio.[60,61] An important distinction of note is that the monkey model is an injury model unlike human primary open-angle glaucoma. Nevertheless, the animal does respond to drugs that affect the TM and provides results that extrapolate to glaucoma patients.

In conclusion, this study in ocular hypertensive monkeys finds that OMDI lowers IOP by increasing both trabecular outflow facility and uveoscleral outflow. This novel selective EP2 receptor agonist could be a new option for the treatment of glaucoma.

Acknowledgments

Author Disclosure Statement

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