A Novel Dual Agonist of EP3 and FP Receptors for OAG and OHT: Safety, Pharmacokinetics, and Pharmacodynamics of ONO-9054 in Healthy Volunteers

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Purpose. The use of a dual prostaglandin E3 (EP3) and prostaglandin F (FP) receptor agonist is a novel approach for the reduction of intraocular pressure (IOP) in open angle glaucoma and ocular hypertension and, as such, ONO-9054 may have benefits over existing therapies. The objectives of this phase I study were to assess the safety, tolerability, systemic pharmacokinetics (PK), and pharmacodynamics (PD) profiles of ONO-9054 (Sepetoprost), the prodrug of ONO-AG-367, in healthy, normotensive adults.

Methods. In this randomized, double-masked, placebo-controlled, single-dose escalating study, 48 male and female healthy volunteers each received a single drop of ONO-9054 0.3, 1.0, 3.0, 10.0, 20.0, or 30.0 μg/mL, or matching placebo in each eye. Blood samples of PK were taken up to 24 hours post dose; ocular and systemic safety, tolerability, and PD assessments were conducted up to approximately 72 hours post dose, and on day 7 at the follow-up visit.

Results. We found ONO-9054 was safe and well tolerated and ONO-AG-367 exhibited dose-dependent systemic PK with rapid elimination. The effect of PD was assessed by reduction in IOP, with the maximum change from baseline in IOP in these normotensive individuals of −28.23% achieved at the 30.0 μg/mL dose at 9 hours post administration.

Conclusions. A single dose of the novel EP3 and FP receptor agonist ONO-9054 was safe and well tolerated in healthy volunteers at doses between 0.3 and 30.0 μg/mL and resulted in a significant reduction in intraocular IOP with maximum reduction at 9 hours post dose. This supports further evaluation of ONO-9054 for the treatment of ocular hypertension and open angle glaucoma. (ClinicalTrials.gov number, NCT01508988.)

Keywords: open-angle glaucoma, ocular hypertension, ONO-9054, sepetoprost, healthy volunteers

Glaucoma is the second most common cause of blindness worldwide.[1] It is a chronic ocular disease characterized by progressive optic neuropathy and visual field loss. Although glaucoma is not defined by raised IOP, elevated IOP is one of the strongest risk factors for both development and progression of glaucoma and is the primary target of treatment.[2] Reducing IOP prevents or delays the onset of open-angle glaucoma in patients with ocular hypertension[3] and slows progression among those with both elevated pressure and normal tension open-angle glaucoma.[4–8]

Two multicentered NIH-sponsored studies, the Ocular Hypertension Treatment Study (OHTS)[8] and the Early Manifest Glaucoma Trial (EMGT),[7] have each independently found that every additional 1 mm Hg of IOP lowering can translate into an approximate 10% decreased risk of glaucomatous development and progression. In addition, the collaborative normal tension glaucoma study identified that a slower rate of incident field loss is seen with reduction in IOP in normal tension subjects.[9] Therefore, the goal of treatment in glaucoma and ocular hypertension (OHT) is to reduce IOP to a target pressure sufficiently low to prevent disease progression.

Prostaglandin analogues (PGAs) are commonly prescribed IOP-lowering medications employed in the treatment of open-angle glaucoma and OHT. Prostaglandin analogues latanoprost, travoprost, and bimatoprost target the prostanoid F (FP) receptor and are thought to lower IOP mainly by increasing outflow of aqueous humor, primarily through the uveoscleral pathway.[10,11] Prostanoid EP3 receptors found in the trabecular meshwork and ciliary muscle[12] have demonstrated the potential to augment the reduction in IOP after the application of FP agonists in monkeys.[13] Existing medications can be suboptimal. Approximately 20% to 30% of patients taking PGAs require some sort of adjunctive therapy to control IOP[14]; the beta blocker timolol is believed to lack nocturnal efficacy[15] and the emerging class of rho kinase inhibitors are associated with hyperemia. Reports of adverse events (AEs) of hyperemia occurred in approximately 50% of subjects. In addition, AR13324 demonstrated a smaller reduction in IOP than latanoprost.[16]

A novel compound with dual EP3 and FP agonist activity,[17] ONO-9054 is a prodrug that is hydrolyzed to its active form ONO-AG-367 by the action of esterases known to be present in the cornea. Unlike currently available PGAs which are high-affinity FP agonists, ONO-9054 has equivalent high agonist activity at both the human EP3 and FP receptors.[17] Preclinical data of ONO-9054 show more potent and longer-lasting IOP-lowering effects in monkeys, suggesting greater efficacy in humans than currently available PGAs[17] and reduction in pressure is via an increase in trabecular outflow (Karakawa T, et al. *IOVS* 2015;56:ARVO E-Abstract 1974) in addition to the increase in uveoscleral outflow expected from PGAs.[10,11]

This single-dose escalation phase 1 clinical trial investigated the safety and tolerability of ONO-9054, a compound that simultaneously stimulates both prostanoid EP3 and FP receptors and may therefore result in a greater reduction of IOP in humans than existing PGAs.

Materials and Methods

The chemical names and structures for ONO-9054 and ONO-AG-367 have previously been described.[17] The study protocol, patient informed consent form, and relevant documents were approved by Independent Investigational Review Board, Inc. prior to starting the study. The nature and possible consequences of participation were explained prior to subjects providing written, informed consent. The study was conducted at the Covance Phase 1 Unit (Dallas, TX, USA) in accordance with Good Clinical Practice (International Conference on Harmonisation, Guidance E6, 1996), United States Code of Federal Regulations, and the tenets of the Declaration of Helsinki. Batches of ONO-9054 were manufactured according to Good Manufacturing Practice at Nitto Medic, Ltd. (Osaka, Japan). We formulated ONO-9054 in an aqueous solution containing 0.25 mg/2.5 mL benzalkonium chloride and packaged in 5-mL polypropylene bottles containing 2.7 mL ophthalmic solution. Placebo and ONO-9054 packaging was identical and both ONO-9054 and placebo were administered by site staff as a clear, colorless single drop of approximately 30 μL into each eye.

Study Design

This double-masked, randomized, placebo-controlled, single-dose escalation study (ClinicalTrials.gov: NCT01508988) was designed to evaluate the safety, tolerability, PK, and PD of ONO-9054 in healthy adult subjects. The design consisted of a screening period of up to 28-days, a 2-day predose period, a 4-day dosing and evaluation period, and a follow-up visit at 7 days post dose. Subjects were confined to the study unit from predose through to the end of the 4 day dosing and evaluation period. A single dose was administered on day 1 of the 4 day dosing and evaluation period.

Forty-eight healthy adult male and female subjects aged 18 to 64 years were evaluated in six cohorts. All subjects had IOP < 21 mm Hg at both the screening and baseline visits with best corrected visual acuity (BCVA) of 20/30 or better. Subjects with past ocular trauma, history of previous intraocular or laser surgery within 3 months, or any condition precluding reliable applanation tonometry were excluded. In addition subjects with current or chronic bronchial asthma or a forced expiratory volume of <80% of predicted value were also excluded. Escalating doses of ONO-9054 were evaluated in six sequential cohorts and within each cohort subjects were randomized to study medication in a 3:1 ratio (active treatment ONO-9054 versus placebo). In each cohort, six subjects received ONO-9054 and two subjects received placebo. In total, 12 subjects were randomized to placebo, and six subjects each were randomized to 0.3, 1.0, 3.0, 10.0, 20.0, and 30.0 μg/mL ONO-9054. Subjects received a single drop of study medication instilled into each eye by site staff. Escalation to the next dose (cohort) was only permitted if adequate safety and tolerability were demonstrated and the results did not meet stopping criteria which included drug-related forced expiration velocity 1 (FEV_1) $< 60\%$ of predicted value, AEs in ≥ 2 subjects or a serious adverse event (SAE). Randomization lists were generated by a Covance statistician and programmer who were independent of the study team.

Safety and Tolerability

Safety assessments included AEs, ocular examination, spirometry, safety laboratory tests (hematology, serum chemistry, and urinalysis), 12-lead and telemetry ECGs, vital signs including respiratory rate, and physical examinations. We recorded AEs from day 1 post dose through the follow-up visit on day 7. Ocular safety was investigated by slit-lamp examination, including assessment of injection, cells and flare, slit-lamp examination with indirect ophthalmoscopy for examination of the fundus, fundus photomicrography, BCVA by Early Treatment Diabetic Retinopathy Study chart, and pupillometry. Fundus imaging was obtained before and after spirometry, to ensure that no retinal vascular changes occurred as a result of either the drug or spirometry. Subjects underwent dilated fundus examinations with infrared fundus photographic imaging (Heidelberg Spectralis; Heidelberg Engineering, Heidelberg, Germany) on day 2 (before spirometry); day 1 predose (after spirometry); and at 5, 10, and 30 minutes, and 2, 5, and 73 hours after administration of study drug. Image analysis was by central reading which was conducted by the same independent, masked reader. Retinal vasculature changes were evaluated by dilated fundus photomicroscopy and indirect ophthalmoscopy examination. Symptoms related to retinal vasoconstriction were assessed (photopsias, zig zag image of light patterns, fortification pattern of lights, and transient obscuration of vision). Indirect ophthalmoscopy was used for evaluation of the retina.

Ocular tolerability was evaluated by subject ratings of ocular discomfort (photophobia, itching, tearing, dryness, and discharge, rated on a scale of 0–4) and investigator rating of conjunctival hyperemia using an ocular redness scale (Ora Calibra #6.0b; used under license from Ora, Inc., Andover, MA, USA). Patients were rated on a scale of 0 to 3 (0–0.5: absent; 1–1.5: mild; 2–2.5: moderate; and 3: severe). Scores were compared to baseline and analysis of hyperemia was based on scores taken from the worst eye.

Pharmacodynamics

Recordings of IOP were made by a masked observer and recorder to avoid bias, using Goldman applanation tonometry. Each IOP reading was the mean of two separate measurements or the median of three readings if the difference between the first two readings was greater than 2 mm Hg. Measurements of IOP were obtained at 23, 21, 19, and 15 hours predose as well as 1, 3, 5, 9, 25, 27, 29, 33, 49, 51, 53, 57, and 73 hours post dose.

Pharmacokinetics

Blood samples were collected to characterize the pharmacokinetic profile of ONO-9054 and its active metabolite ONO-AG-367. Blood PK samples were collected via venipuncture at predose, 5, 10, 20, 30, 45 minutes, and 1, 1.5, 2, 4, 6, 8, 12, and 24 hours post dose. These samples were processed to plasma

Table 1. Demographics and Baseline Characteristics by Treatment Group

Parameter	Placebo, $n = 12$	ONO-9054, µg/mL					
		0.3, $n = 6$	1.0, $n = 6$	3.0, $n = 6$	10.0, $n = 6$	20.0, $n = 6$	30.0, $n = 6$
Mean age, y (SD)	36.5 (9.6)	30.5 (10.5)	39.3 (9.5)	34.5 (14.2)	38.3 (5.2)	26.3 (8.9)	40.2 (7.7)
Sex, *n* (%)							
Male	10 (83.3)	4 (66.7)	5 (83.3)	5 (83.3)	2 (33.3)	4 (66.7)	5 (83.3)
Female	2 (16.7)	2 (33.3)	1 (16.7)	1 (16.7)	4 (66.7)	2 (33.3)	1 (16.7)
Race, *n* (%)							
Caucasian	4 (33.3)	4 (66.7)	2 (33.3)	2 (33.3)	0	4 (66.7)	2 (33.3)
Black	6 (50.0)	2 (33.3)	4 (66.7)	4 (66.7)	6 (100)	2 (33.3)	4 (66.7)
Other	2 (16.7)	0	0	0	0	0	0
Ethnicity, *n* (%)							
Not Hispanic or Latino	11 (91.7)	4 (66.7)	4 (66.7)	5 (83.3)	6 (100)	4 (66.7)	4 (66.7)
Hispanic or Latino	1 (8.3)	2 (33.3)	2 (33.3)	1 (16.7)	0	2 (33.3)	2 (33.3)
IOP average of two eyes, mean mm Hg (SD)							
08:00	12.9 (2.0)	14.0 (3.0)	12.5 (2.7)	13.3 (2.1)	13.9 (0.9)	13.3 (2.2)	12.5 (2.5)
10:00	12.9 (2.0)	13.3 (2.3)	13.0 (1.5)	14.3 (2.5)	13.5 (1.6)	12.9 (2.5)	11.5 (1.7)
12:00	12.8 (1.9)	14.2 (2.2)	12.4 (1.8)	13.7 (2.0)	13.0 (0.9)	12.6 (1.1)	11.0 (1.7)
16:00	13.4 (2.0)	15.2 (3.0)	11.5 (1.7)	13.8 (2.6)	13.8 (1.2)	13.1 (1.3)	11.6 (1.3)
BCVA letter score							
Left eye	90.9	87.0	89.7	87.0	87.0	88.8	89.0
Right eye	90.0	84.7	85.0	90.5	86.7	88.5	91.2
FEV_1 mean % of predicted (SD)	101.8 (8.6)	104.3 (13.1)	104.5 (12.2)	96.8 (14.0)	103.8 (10.2)	93.8 (6.6)	97.2 (10.8)

and stored at −70°C or below. The plasma levels of ONO-9054 and ONO-AG-367 were determined using a validated high-performance liquid chromatography-tandem mass spectrometer (LC/MS/MS) method with a quantitative range of 5 to 5000 pg/mL and 1 to 200 pg/mL, respectively. Each plasma sample was added with internal standard (deuterated ONO-9054 or ONO-AG-367); analytes were isolated through solid phase extraction and eluted with acetonitrile or acetonitrile/formic acid (100:1, vol/vol). The eluate was evaporated under a nitrogen stream and the remaining residue was reconstituted with acetonitrile/{water/acetic acid (1000:0.1, vol/vol)} (1:4, vol/vol). The resulting samples were injected into the LC/MS/MS system.

The following plasma pharmacokinetic parameters for ONO-AG-367 were calculated using a noncompartmental model (Phoenix WinNonlin, ver. 6.1; Certara, St. Louis, MO, USA): maximum measured concentration (Cmax), time to Cmax (Tmax), elimination half-life (T1/2), area under the plasma concentration versus time curve (time 0 to last quantifiable concentration, AUClast), area under the plasma concentration versus time curve (time 0 to infinity, AUCinf). Dose proportionality of Cmax and AUClast were estimated with the criterion of whether or not the 95% confidence interval (CI) of the slope (β) included 1.0, using the power model. The point estimate of the intercept ($\ln(\alpha)$) and β with 95% CI was calculated by linear regression according to the least squares method.

Statistical Analysis

The sample sizes for this study were chosen based upon practical considerations. No a priori statistical assumptions were made. The statistical analysis was performed using statistical analysis software (SAS, ver. 9.2; SAS Institute, Cary, NC, USA). Demographic data including ocular history; physical and ocular examinations; PK; PD; spirometry; vital signs; 12-lead and telemetry ECG; and safety laboratory evaluations (hematology, serum chemistry, and urinalysis) were summarized using descriptive statistics.

All subjects who received the study medication were included in the safety and PD analysis set. The analysis population of PK included all enrolled subjects who received ONO-9054 and provided sufficient samples for pharmacokinetic evaluations.

Results

Demographics

Forty-eight subjects were enrolled and randomized; 36 received a single dose of ONO-9054 at concentrations of 0.3 to 30.0 µg/mL, and 12 subjects received placebo. The majority of subjects were black (28/48, 58.3%), with 18/48 (37.5%) Caucasian subjects. The study enrolled more males, 35/48 (72.9%), than females and ages ranged from 18 to 53 years. Other demographic and baseline characteristics were not notably different across treatment groups. The baseline demographic data are presented in Table 1.

Safety and Tolerability

There were no deaths or SAEs during the study. No subjects withdrew from the study due to AEs and all reported AEs had resolved by the end of the study. One subject who received placebo in cohort 2 withdrew consent, completed all assessments through day 4, but did not complete the study. Overall, similar numbers of AEs were reported in subjects who received ONO-9054 and placebo (Table 2) with 10 AEs (all doses combined) reported in 8 of the 36 (22.2%) subjects who received ONO-9054 and 5 AEs in 3 of the 12 (25.0%) subjects who received placebo (Table 2). Out of the 17 AEs in the study, 16 were rated as mild. Only one (influenza) was rated as moderate and this was experienced by a subject randomized to receive placebo. The most frequently reported

Table 2. Adverse Events by Treatment Group Following a Single Ocular Instillation of Ono-9054 to Both Eyes

System Organ Class* Preferred Term	Placebo, *n* = 12, *n* (%)	0.3 µg/mL, *n* = 6, *n* (%)	1.0 µg/mL, *n* = 6, *n* (%)	3.0 µg/mL, *n* = 6, *n* (%)	10.0 µg/mL, *n* = 6, *n* (%)	20.0 µg/mL, *n* = 6, *n* (%)	30.0 µg/mL, *n* = 6, *n* (%)
Total number of AEs	**5**	**1**	**2**	**1**	**2**	**2**	**2**
Number of subjects with at least one AE, *n* (%)	**3 (25.0)**	**1 (16.7)**	**2 (33.3)**	**1 (16.7)**	**1 (16.7)**	**1 (16.7)**	**2 (33.3)**
Eye disorders	**0**	**0**	**0**	**1 (16.7)**	**0**	**0**	**1 (16.7)**
Eye irritation	0	0	0	1 (16.7)	0	0	0
Photopsia	0	0	0	0	0	0	1 (16.7)
Gastrointestinal disorders	**1 (8.3)**	**0**	**0**	**0**	**0**	**0**	**0**
Nausea	1 (8.3)	0	0	0	0	0	0
General disorders and administration site conditions	**0**	**0**	**0**	**0**	**0**	**1 (16.7)**	**0**
Vessel puncture site anesthesia	0	0	0	0	0	1 (16.7)	0
Immune system disorders	**1 (8.3)**	**0**	**0**	**0**	**0**	**0**	**0**
Perennial allergy	1 (8.3)	0	0	0	0	0	0
Infections and infestations	**2 (16.7)**	**0**	**0**	**0**	**0**	**0**	**0**
Genital herpes	1(8.3)	0	0	0	0	0	0
Influenza	1(8.3)	0	0	0	0	0	0
Injury, poisoning and procedural complications	**0**	**0**	**0**	**0**	**0**	**0**	**1 (16.7)**
Ligament sprain	0	0	0	0	0	0	1 (16.7)
Nervous system disorders	**1 (8.3)**	**1 (16.7)**	**2 (33.3)**	**0**	**0**	**1 (16.7)**	**0**
Dizziness	1 (8.3)	0	0	0	0	0	0
Headache	0	1 (16.7)	1 (16.7)	0	0	1 (16.7)	0
Presyncope	0	0	1 (16.7)	0	0	0	0
Skin and subcutaneous tissue disorders	**0**	**0**	**0**	**0**	**1 (16.7)**	**0**	**0**
Dermatitis acneiform	0	0	0	0	1 (16.7)	0	0
Pruritus	0	0	0	0	1 (16.7)	0	0

Bold text indicates system organ class and non–bold text indicates preferred term.

AE was mild headache. Of the three AEs considered possibly related to drug, two of these events (dizziness and nausea) were reported in subjects who received placebo. One subject reported mild, transient, photopsia 5 minutes after the instillation of a 30.0-µg/mL dose which resolved by 10 minutes post dose without any treatment. There were no retinal findings suggestive of retinovascular constriction in this subject.

The only findings from ocular safety assessments were conjunctival injection. No treatment-related (ONO-9054 versus placebo) or dose-related trends were observed in ocular safety findings, clinical laboratory results, spirometry, vital sign measurements, 12-lead ECG findings, or telemetry results.

When present, mild hyperemia had an onset between 2 and 5 hours after dose and was consistent with conjunctival injection as determined by biomicroscopy. Generally, hyperemia scores increased from a score of 0 to 0.5 (no hyperemia) at baseline with each ascending dose. On day 1, a maximum score of 1 to 1.5 (mild) was recorded in 3 of 12 subjects (25%) after dosing with placebo and 4 of 6 subjects (66.7%) after a dose of 3.0 µg/mL. The highest observed score of 3 (severe) was recorded in one subject (16.7%) after a dose of 30.0 µg/mL. The distribution of maximum hyperemia scores from days 1 to 4 by treatment group is summarized in Table 3. No hyperemia or injection was considered by investigators to be clinically significant. The mean hyperemia symptom score (average of two eyes) is presented by postdose time point and treatment group in Figure 1, where hyperemia was seen, it returned to baseline by 73 hours post dose in all groups except the 30-µg/mL group. The majority of subjects reported the tolerability measures as 0 (absent). The few responses in other subjects receiving ONO-9054 were all rated as 1 (mild). Itching was the most frequently reported symptom with similar frequencies in the placebo (1/12 [8.3%]) and ONO-9054 dose

Table 3. Distribution of Maximum Hyperemia Score From Days 1 to 4 by Treatment Group*

		ONO-9054					
Maximum Score	Placebo, *n* = 12, *n* (%)	0.3 µg/mL, *n* = 6, *n* (%)	1.0 µg/mL, *n* = 6, *n* (%)	3.0 µg/mL, *n* = 6, *n* (%)	10.0 µg/mL, *n* = 6, *n* (%)	20.0 µg/mL, *n* = 6, *n* (%)	30.0 µg/mL, *n* = 6, *n* (%)
0, 0.5	9 (75.0)	5 (83.3)	6 (100)	2 (33.3)	4 (66.7)	0	0
1, 1.5	3 (25.0)	1 (16.7)	0	4 (66.7)	1 (16.7)	4 (66.7)	4 (66.7)
2, 2.5	0	0	0	0	1 (16.7)	2 (33.3)	1 (16.7)
3	0	0	0	0	0	0	1 (16.7)

0–0.5, no hyperemia; 1–1.5, mild hyperemia; 2–2.5, moderate hyperemia; 3, severe hyperemia.
* When two eyes in the same subject had different results, the worse (more severe grade) was used.

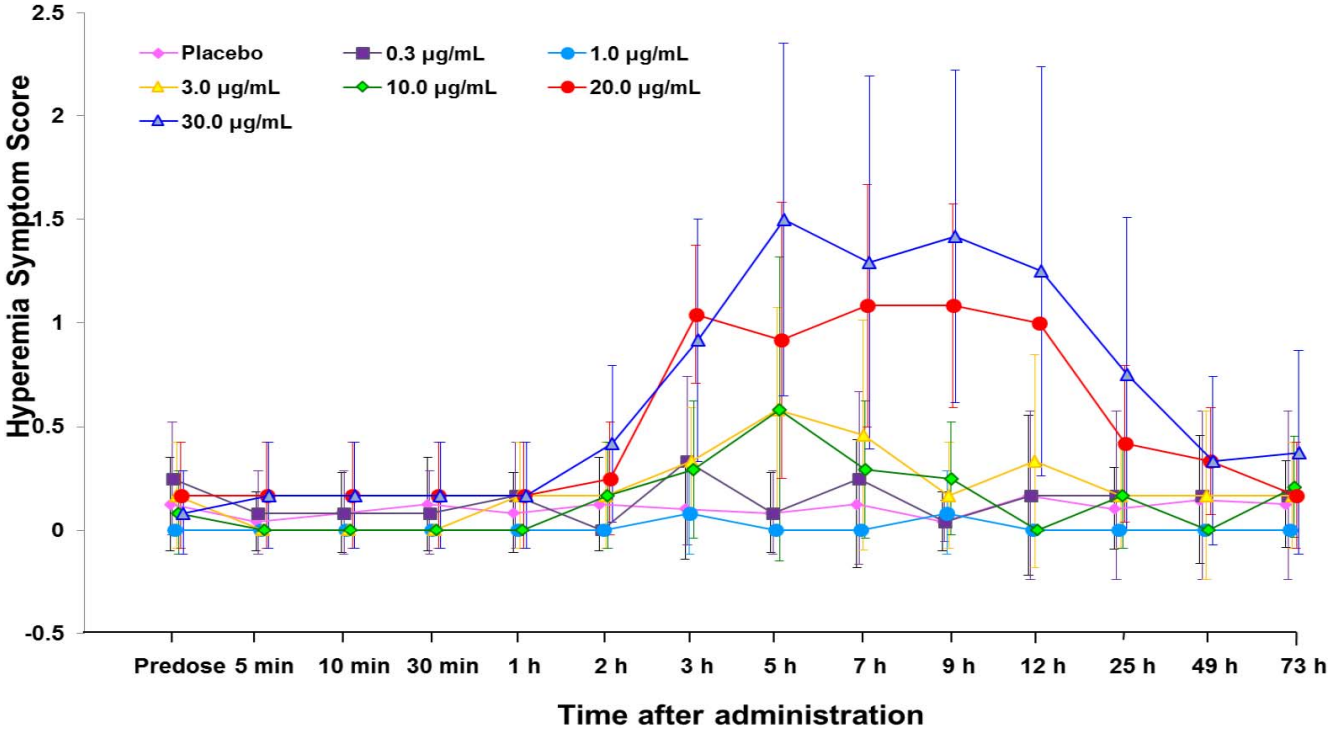

FIGURE 1. Mean hyperemia symptom score (average of two eyes) with SDs by postdose time point and treatment group following a single dose of ONO-9054 0.3 (*purple squares*), 1.0 (*cyan circles*), 3.0 (*yellow triangles*), 10.0 (*green diamonds*), 20.0 (*red circles*), or 30.0 µg/mL (*blue triangles*), or placebo (*pink diamonds*). Hyperemia was assessed using the ocular redness scale (Ora, Inc.) as 0 to 0.5, absent; 1 to 1.5, mild; 2 to 2.5, moderate; and 3, severe.

groups (2/36 [5.6%]). There were no apparent dose-responses for any of the five eyedrop tolerability parameters evaluated (photophobia, itching, tearing, dryness, and discharge; Table 4). We found ONO-9054 administered in single doses from 0.3 to 30.0 µg/mL appeared to be safe and well tolerated in healthy adult male and female subjects.

Pharmacodynamics (Ocular Hypotensive Effect)

The mean baseline values for IOP in healthy adult subjects ranged from 11.0 to 15.2 mm Hg with no notable differences across treatment groups. The reduction of IOP with respect to baseline became more pronounced with ascending doses of

TABLE 4. Maximum Severity in Eyedrop Tolerability from Day 1 to Day 4 by Treatment Group Following a Single Ocular Instillation of ONO-9054 to Both Eyes*

		ONO-9054					
Tolerability Parameter	Placebo, *n* = 12, *n* (%)	0.3 µg/mL, *n* = 6, *n* (%)	1.0 µg/mL, *n* = 6, *n* (%)	3.0 µg/mL, *n* = 6, *n* (%)	10.0 µg/mL, *n* = 6, *n* (%)	20.0 µg/mL, *n* = 6 *n* (%)	30.0 µg/mL, *n* = 6, *n* (%)
Photophobia							
0	12 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)
Itching							
0	11 (91.7)	6 (100)	6 (100)	5 (83.3)	5 (83.3)	6 (100)	6 (100)
1	0	0	0	1 (16.7)	1 (16.7)	0	0
2	1 (8.3)	0	0	0	0	0	0
Tearing							
0	11 (91.7)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)
1	1 (8.3)	0	0	0	0	0	0
Dryness							
0	11 (91.7)	6 (100)	6 (100)	6 (100)	5 (83.3)	6 (100)	6 (100)
1	1 (8.3)	0	0	0	1 (16.7)	0	0
Discharge							
0	12 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)	6 (100)

0, absent; 1, mild; 2, moderate; 3, severe with stinging or burning; 4, severe with blurred or dimmed vision.
* When two eyes in the same subject had different results, the worse (more severe grade) was used.

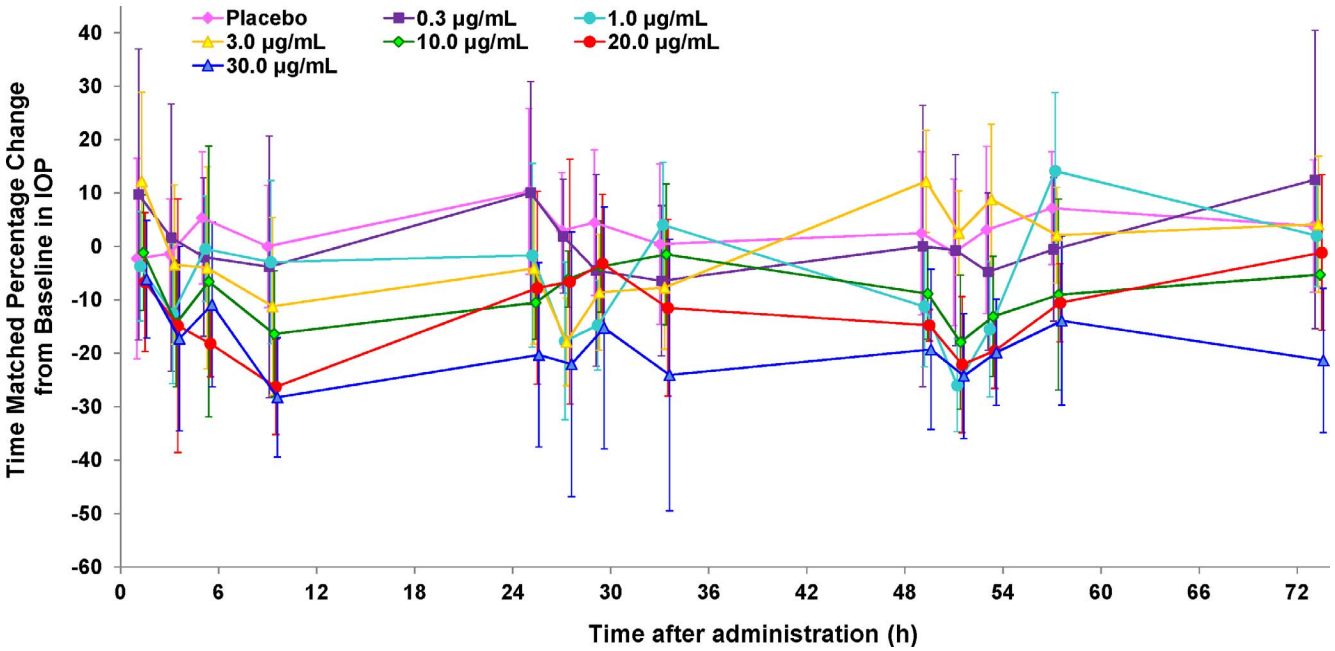

FIGURE 2. Intraocular pressure mean percentage change from baseline (average of two eyes) by scheduled time point following a single dose of ONO-9054 0.3 (*purple squares*), 1.0 (*cyan circles*), 3.0 (*yellow triangles*), 10.0 (*green diamonds*), 20.0 (*red circles*) or 30.0 µg/mL (*blue triangles*), or placebo (*pink diamonds*).

ONO-9054 (Fig. 2). Mean change and mean percentage change from baseline across dose groups showed a reduction in IOP at most postdose time points for ONO-9054 doses of 10.0, 20.0, and 30.0 µg/mL, with maximum changes from baseline in mean IOP (change from baseline) observed at 9 hours post dose; 9.58 mm Hg (−3.5 mm Hg) and 8.29 mm Hg (−3.3 mm Hg) in the 20.0 and 30.0 µg/mL dose groups, respectively; corresponding to a mean percentage change from baseline of −26.3% and −28.2%, respectively. There was no consistent reduction in IOP in the placebo group (i.e., mean percentage change from baseline in IOP in the placebo group was positive at most time points).

Pharmacokinetics

All plasma concentrations of ONO-9054 were below the limit of quantitation (BLQ; <5.00 pg/mL) over the dose range tested except for a single time point in one subject who received 30.0 µg/mL ONO-9054. Therefore, no plasma PK parameters were calculated for ONO-9054. The free acid metabolite of ONO-9054, ONO-AG-367, was detectable in all subjects except at the lowest dose (Table 5; Fig. 3). Following a single dose, mean plasma concentrations of ONO-AG-367 reached Cmax within 10 to 15 minutes based on median Tmax values and then declined rapidly with a mean T1/2 of 0.48 to 0.83 hours; the plasma concentration of ONO-AG-367 was BLQ (<1.00 pg/mL) at 6 hours and later time points. Since AUCinf at lower doses (3.0 µg/mL or less) could not be calculated due to the lack of detectable plasma concentrations, dose proportionality of AUCinf was not evaluated. Maximum measured concentration of ONO-AG-367 increased in a dose-proportional manner over the dose range studied (Fig. 4A) with the point estimate (95% CI) of β (1.02 [0.81–1.23]). We found AUClast of ONO-AG-367 increased in an approximately dose-proportional manner from 0.3 to 10.0 µg/mL with the point estimate (95% CI) of β of 1.54 (0.95–2.13), but in a more than dose-proportional manner above 10.0 µg/mL (0.3–20.0 µg/mL: 1.48 [1.17–1.79], 0.3 to 30.0 µg/mL: 1.47 [1.24–1.70]) Figure 4B.

TABLE 5. Plasma PK Parameters for ONO-AG-367 Following a Single Ocular Instillation of ONO-9054 to Both Eyes

	ONO-9054					
PK Parameters	0.3 µg/mL, $n = 6$	1.0 µg/mL, $n = 6$	3.0 µg/mL, $n = 6$	10.0 µg/mL, $n = 6$	20.0 µg/mL, $n = 6$	30.0 µg/mL, $n = 6$
AUCinf, pg·h/mL	BLQ	BLQ	BLQ	6.09 (2.17)*	10.6 (2.03)	21.4 (6.82)†
AUClast, pg·h/mL	BLQ	0.00706 (NC)	0.703 (0.396)	3.88 (2.21)	9.34 (1.96)	18.1 (6.61)
Cmax, pg/mL	BLQ	0.170 (NC)	2.02 (1.10)	5.54 (2.71)	16.5 (6.93)	29.1 (9.63)
Tmax, h	NC	0.17 (0.17, 0.17)‡	0.17 (0.17, 0.33)†	0.25 (0.17, 0.33)	0.17 (0.17, 0.17)	0.17 (0.083, 0.33)
T1/2, h	NC	NC	NC	0.48 (0.20)§	0.69 (0.12)	0.83 (0.31)

All data except for Tmax are shown as the means and SDs. Values of Tmax are shown as the medians and ranges. NC, not calculated.
* $n = 3$.
† $n = 5$.
‡ $n = 1$.
§ $n = 4$.

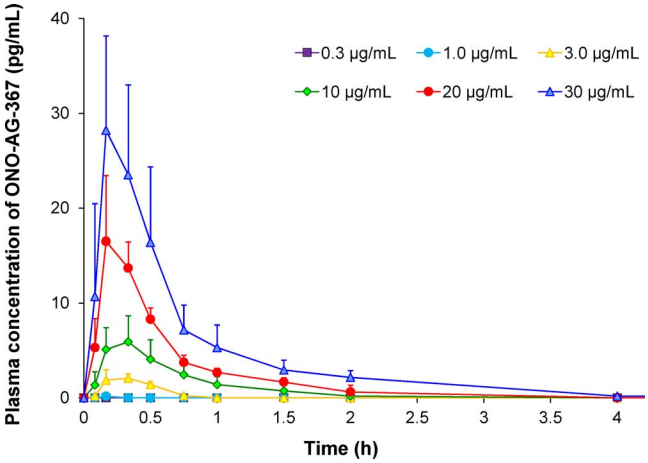

FIGURE 3. Plasma concentration–time plots for the active metabolite ONO-AG-367 following a single dose of ONO-9054 0.3 (*purple squares*), 1.0 (*cyan circles*), 3.0 (*yellow triangles*), 10.0 (*green diamonds*), 20.0 (*red circles*), or 30.0 µg/mL (*blue triangles*).

DISCUSSION

ONO-9054 is a novel, dual EP3 and FP receptor agonist with the potential to provide a greater reduction in IOP than existing PGA therapies. ONO-9054 is a prodrug that is hydrolyzed to its active prostaglandin receptor agonist form, ONO-AG-367, by esterases which are present in the cornea and other tissues. Preclinical data suggest that the efficacy of ONO-9054 in reducing IOP may be greater than that of prostaglandin analogues.[17] ONO-9054 enhances aqueous outflow via both uveoscleral and conventional pathways (Karakawa T, et al. *IOVS* 2015;56:ARVO E-Abstract 1974). Adjunctive therapy is known to be required by approximately 20% to 30% of patients receiving existing PGAs in order to control IOP[14] and it is anticipated that a drug with a dual mechanism, effecting two outflow pathways may reduce the need for additional therapies.

Since this was a first in human study of a novel dual EP3 and FP receptor agonist and in safety pharmacology studies in monkeys, transient increases in respiratory rate and decreases in tidal volume were observed following intravenous administration of ONO-9054 (data not presented), subjects with respiratory conditions were excluded and spirometry and respiratory rate measurements were taken to ensure subjects' safety. In addition, particular notice was taken of ECG, telemetry, and vital signs results. There were no changes in FEV_1 or respiration rate following a single dose of ONO-9054 at any concentration, which may relate to the low systemic levels of the active metabolite and is consistent with the absence of respiratory effects in asthmatics treated with latanoprost[18] and there were no changes in cardiac parameters. Moreover clearance of ONO-AG-367 from the plasma was relatively rapid after dose and the plasma concentration of ONO-AG-367 was BLQ (<1.00 pg/mL) at 6 hours and later time points. A single drop of ONO-9054 was safe and well tolerated in healthy volunteers at all of the tested dose levels, with no apparent dose response in incidence or intensity of AEs and a similar incidence to the placebo group. Prostaglandin analogues are associated with ocular adverse effects, which are typically observed after 3 to 6 months of dosing, with iris pigmentary changes, eyelash growth, periocular pigmentary changes, and prostaglandin-associated periorbitopathy.[19,20] It is not possible to predict from a single dose what the likelihood is of these effects occurring from long-term administration with ONO-9054; this would be evaluated in longer, repeat dose studies.

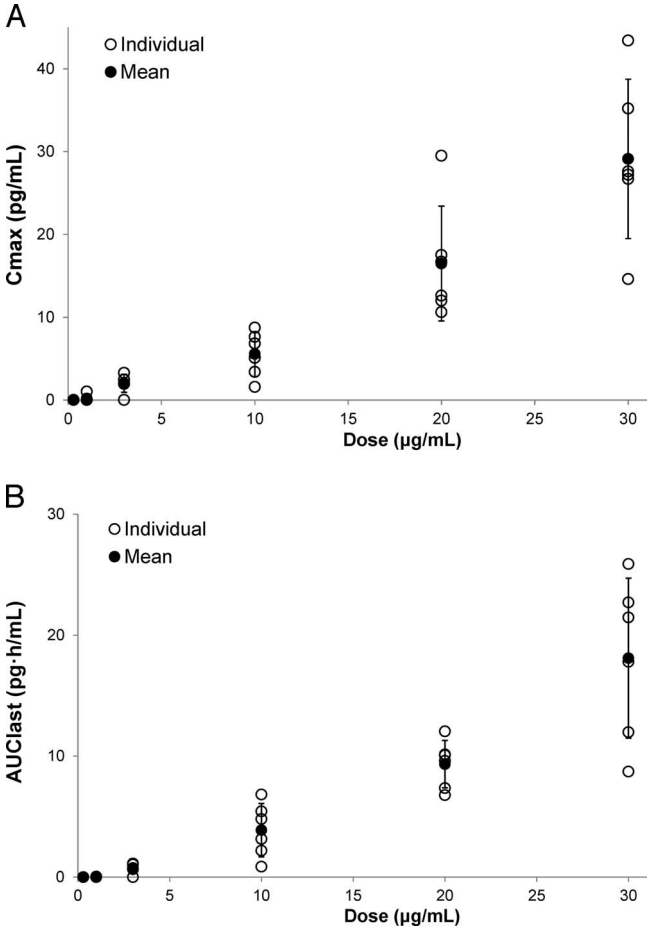

FIGURE 4. Relationship between plasma Cmax (**A**) or AUClast (**B**) and dose for both individual (*open circles*) and mean (*closed circles*) data for the active metabolite ONO-AG-367 following a single dose of ONO-9054.

In preclinical studies, retinal vasculature effects had been observed in albino rats (data not shown). The relevance of these findings to humans was unclear and therefore retinal vasculature effects in humans were examined in this study, using a central reader to standardize results. There were no changes in retinal vasculature evident and no changes in ocular safety parameters recorded. A single subject experienced symptoms potentially related to retinal vasoconstriction which was mild, transient photopsias without any retinal vascular changes observed clinically by direct and indirect ophthalmoscopy or through measurement of the retinal vessel caliber from serial fundus imaging.

Mild hyperemia (graded 1–1.5 on the ocular redness scale; Ora, Inc.) that appeared dose related was observed around 2 to 5 hours after dosing and was consistent with the bulbar conjunctival injection recorded by slit lamp examination, although it is of interest that hyperemia was also reported in the placebo group and so may, in part, relate to the formulation. Hyperemia is a known effect of prostaglandin analogues and is expected to be less severe than that seen with Rho Kinase inhibitors.[16] The low level of hyperemia may be beneficial with respect to medication compliance in patients.[20] Mild itching was the most frequently reported tolerability parameter, with a single case of dryness reported.

Intraocular pressure was evaluated as a PD measure for ONO-9054. Mean change and mean percentage change from baseline in IOP across dose groups showed a reduction in IOP

at most postdose time points at ONO-9054 single doses of 10.0, 20.0, and 30.0 μg/mL. Measurements at 3 to 9 hours post dose inclusive suggest a dose-related trend, although the numbers of subjects in each dose group ($n = 6$) were too small for a definitive assessment of dose response. There was no consistent reduction in IOP in the placebo group. Intraocular pressure lowering in normotensive healthy volunteers after a single dose, with a maximum reduction of 28% at 9 hours post dose, is indicative of possible utility in normotension glaucoma subjects as well as patients with elevated intraocular pressure. In addition, ONO-9054 has an effect on reduction in IOP that is sustained over greater than 24 hours in healthy volunteers, suggestive that a once daily dosing routine would be feasible.

The prodrug ONO-9054 was rapidly converted after ocular instillation into the active dual EP3 and FP receptor agonist ONO-AG-367. ONO-AG-367 had predictable pharmacokinetics which showed a dose-dependent increase in plasma concentrations. The pharmacodynamic effect continued for greater than 24 hours post dose as has previously been shown in monkeys[17]; it is not yet known why this sustained effect occurs.

Although the IOP-lowering effect of drugs in healthy volunteers is not always comparable to that which might be observed in ocular hypertensive or glaucoma patients, preliminary studies of ocular PD are valuable for setting dose ranges and tolerability parameters in future studies of elevated IOP in the glaucoma and ocular hypertension populations. These results support additional clinical trials to demonstrate efficacy, safety, and pharmacokinetics of multiple doses of ONO-9054 ophthalmic solution to manage patients with normal tension glaucoma as well as elevated pressures as a result of glaucoma or ocular hypertension.

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