EP3/FP dual receptor agonist ONO-9054 administered morning or evening to patients with open-angle glaucoma or ocular hypertension: results of a randomised crossover study

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ABSTRACT
Background/aims The novel prostaglandin E (EP) 3 and prostaglandin F (FP) receptor agonist ONO-9054 is effective in lowering intraocular pressure (IOP) in patients with ocular hypertension and open-angle glaucoma when administered once daily. This study compares the effects of morning (AM) versus evening (PM) dosing of ONO-9054 on tolerability and IOP lowering.
Methods This was a single-centre, randomised, double-masked, two-sequence, placebo-controlled crossover study in 12 subjects with bilateral primary open-angle glaucoma or ocular hypertension. Two 14-day crossover regimens were separated by a 2-week washout: ONO-9054 (1 drop to each eye) in the morning (07:00) and vehicle in the evening (19:00) and vice versa. IOP was measured multiple times during select days. Ocular examinations also evaluated safety and tolerability.
Results Mild ocular hyperaemia, reported by six subjects with PM dosing, was the most frequent adverse event. Mild to moderate dryness was also slightly more frequent after PM dosing. Maximum IOP reduction from baseline occurred on day 2 with decreases from baseline of −7.4 mm Hg (−30.8%) for AM dosing and −9.1 mm Hg (−38.0%) for PM dosing; after 14 days, mean reduction in IOP was −6.8 mm Hg (−28.6%) for AM dosing and −7.5 mm Hg (−31.0%) for PM dosing.
Conclusions PM dosing of ONO-9054 was associated with a slightly increased frequency of mild hyperaemia and mild to moderate dryness. Both dosing schedules provided sustained reduction in IOP.
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INTRODUCTION
Glaucoma is an insidious progressive optic neuropathy that often causes irreversible ganglion cell damage leading to permanent vision loss. The objective of glaucoma management is to preserve visual function by providing significant and sustained decrease in intraocular pressure (IOP) by means of pharmaceuticals, office-based laser procedures, minimally invasive glaucoma surgery and conventional surgical procedures.1–3

Pharmacological therapy for ocular hypertension (OHT) and glaucoma include several classes of drugs. Prostaglandin analogues (PGAs) reduce IOP by targeting the prostaglandin F (FP) receptor to increase outflow of aqueous humour, primarily through the uveoscleral pathway.4 In the USA, latanoprost, bimatoprost and travoprost are the most commonly prescribed PGAs used to target the FP receptor.5 Although current PGAs are considered the ‘gold standard’ for pharmaceutical reduction of IOP, new classes of PGA molecules with enhanced tolerability and additional therapeutic benefits are being evaluated. One area of investigation is prostaglandin E (EP) receptor agonists. The EP3 receptor is found in the trabecular meshwork and ciliary muscle,6 and has been demonstrated to augment reduction in IOP following the application of EP agonists in monkeys.7

Prodrug ONO-9054 is an isopropyl ester derivative of the biologically active free acid ONO-AG-367 and is a highly selective and potent agonist of both prostaglandin EP3 and FP receptors in vitro.8 Due to its dual receptor activity, the drug has potential to produce a more potent reduction of IOP than drugs that target the FP receptor.8

Although variable, IOP is often more elevated in the early morning hours.3–11 Thus, therapeutic efficacy of topical glaucoma medications should be effective at controlling IOP during this time. The objective of this crossover study was to assess the tolerability and the effect of morning (AM) versus evening (PM) dosing on IOP lowering of ophthalmic solution ONO-9054 in patients diagnosed with primary open-angle glaucoma (OAG) or OHT.

MATERIALS AND METHODS
Subjects
Twelve subjects with a confirmed diagnosis of bilateral OHT or chronic OAG aged 18–80 years were enrolled. Inclusion criteria included an IOP ≥22 mm Hg at 08:00 and ≥21 mm Hg at 10:00 in at least one eye, with ≤35 mm Hg at all measurements in both eyes on the 2 days preceding dosing (day −2 and day −1; 08:00 and 10:00). A best correct visual acuity (BCVA) of at least 20/100, measured by Logarithm of Minimum Angle of Resolution (LogMAR=0.70 or better) was required at screening and on day 1. Other inclusion criteria included central corneal thickness of 500–600 μm at screening in both eyes, ocular cup-to-disc ratio ≤0.8 in both eyes and absence of visual field loss within the previous 6 months. All subjects gave written, informed consent and agreed to washout of all ocular drugs prior to the study.

Excluded from the study were subjects with history of severe ocular trauma in either eye, intraocular or ocular laser surgery within the previous 3 months, refractive surgery within the...
were administered as clear colourless liquid ophthalmic solution to either treatment. The medication coordinator dispensed the alternate dosing schedule.

Sequence 2: After washout, each subject group crossed over to active drug in the evening and placebo in the morning (07:00 to 22:00) and placebo (vehicle) solution in the evening (07:00 to 12:00) and 5, 10, 20, 30 and 45 min and 1, 1.5, 2, and 3 h after dosing. Plasma ONO-9054 and ONO-AG-367 concentrations were determined using validated liquid chromatography/tandem mass spectrometry methods at Quintiles BioSciences. Plasma concentration versus time data were analysed using Phoenix WinNonlin V6.2.1, using a standard non-compartmental model. The following parameters were derived: maximum observed concentration (Cmax), time to Cmax (Tmax) and elimination half-life (T½).

Statistical considerations
Sample size was based on practical considerations. The Safety Set comprised all subjects enrolled in the study who received at least one drop of study medication of any strength (including placebo) instilled in either eye and was used for analysis of pharmacodynamic end points. The Pharmacokinetic Analysis Population included all enrolled subjects who received ONO-9054 and had at least one postdose pharmacokinetic sample.

RESULTS
Disposition and demographic characteristics
Twelve subjects were enrolled and randomised; all completed the study as planned. Each subject received a cumulative total of 28 doses of ONO-9054 30 μg/mL and 28 doses of placebo in each eye. The study population was predominantly white (10/12) and male (8/12) with a mean age of 68.3 years (range: 50–79 years (table 1)). Half of subjects had been treated previously for glaucoma drug therapy. The hyperaemia was rated as none to mild (Grade 1), moderate (Grade 2) or severe (Grade 3). There were no episodes of photophobia, tearing or discharge. There was no apparent difference in frequency between AM and PM dosing.

Safety and tolerability
There were no episodes of photophobia, tearing or discharge. Itching was mild and transient, occurring in two subjects during PM dosing and one subject during AM dosing; there was no apparent difference in frequency between AM and PM dosing. Dryness was mild-moderate occurring in three subjects during PM dosing and one subject during AM dosing.

Hyperaemia was absent to normal in all subjects at baseline. Peak hyperaemia scores during each 14-day sequence were Grade 2 (moderate) for two subjects (16.7%) in the pooled AM dose group, Grade 3 (severe) for one subject (8.3%) in the PM dose group (table 2). Peak scores were recorded 15 h post dose in the AM group and 13 h post dose in the PM group. Mean scores decreased with continued dosing in both groups: after 14 days of dosing, hyperaemia was rated as none to mild (Grade ≤1) for 10/12 (83.3%) subjects in the AM dose group and 9/12 (75.0%) in the PM dose group.

Overall, seven subjects reported 13 adverse events: six subjects reported 11 adverse events in the PM group and two subjects reported three adverse events in the AM group. Common adverse events included itching, tearing, dryness, discharge and sensation of foreign body. Further details of adverse events are provided in Appendix S1.

Pharmacokinetics
ONO-9054 and ONO-AG-367 concentrations were determined using validated liquid chromatography/tandem mass spectrometry methods at Quintiles BioSciences. Plasma concentration versus time data were analysed using Phoenix WinNonlin V6.2.1, using a standard non-compartmental model. The following parameters were derived: maximum observed concentration (Cmax), time to Cmax (Tmax) and elimination half-life (T½).
Any other ocular assessments (slit-lamp examination, BCVA, PM dosing sequence.

There were no ocular adverse events among subjects in the AM group. Six subjects experienced eye disorders in the PM group reported ocular hyperaemia, which occurred during the first week of dosing. All adverse events were judged mild.

The most frequent adverse events reported were hyperaemia and dry eye. Hyperaemia was only recorded as an adverse event if there were any additional clinical changes, or if the condition was reported by the subject. Six subjects (50.0%) in the PM group reported ocular hyperaemia, which occurred during the first 14 days of dosing. All adverse events were judged mild. There were no ocular adverse events among subjects in the AM dosing sequence; six subjects experienced eye disorders in the PM dosing sequence.

No clinically significant progressive changes were noted in any other ocular assessments (slit-lamp examination, BCVA, pupillometry, pachymetry, indirect ophthalmology, cup-to-disc ratio, fundus imaging, impression cytology and visual field) or in clinical laboratory evaluations, vital signs or 12-lead electrocardiographs.

**Pharmacodynamics**

Visual inspection of time plots showed that the initial rate of decline in IOP was greater with AM dosing in both treatment sequences (figure 1). Greater diurnal fluctuation was observed during the first week of AM dosing than PM dosing in both sequences than in following weeks; a cumulative effect produced a dampening of IOP fluctuation during the fourth week of treatment, independent of AM or PM dosing. Observed values of IOP were similar for the pooled AM and pooled PM dose groups at baseline and decreased from 08:00 to the final measurement of the day at 22:00.

Maximum reduction in IOP for pooled data was observed at 08:00 on the second day of dosing in each sequence (day 2 or 30) \(-7.4 \text{ mm Hg (30.8\% from baseline)}\) for AM dosing (1 h post dose) and \(-9.1 \text{ mm Hg (38.0\%) for PM dosing (12 h post dose)}\). After 14 days of dosing, reductions in IOP were similar to those observed on day 2. On day 14, in pooled results from both AM sequences, mean IOP was 16.4 mm Hg and reduction relative to baseline at 08:00 was \(-6.8 \text{ mm Hg (28.6\%)}.\) Twenty-four hours post dose on day 15 at 08:00, reduction relative to baseline was \(-6.7 \text{ mm Hg (27.5\%)}\). In pooled results from both PM sequences after 14 days of dosing.

### Table 2: Maximum hyperaemia score according to dosing sequences

| Ocular hyperaemia | Sequence 01:00 (n=6) | Sequence 02:00 (n=6) | Sequence 13:00 (n=6) | Sequence 14:00 (n=6) | Pooled AM (n=12) | Pooled PM (n=12) |
|-------------------|----------------------|----------------------|----------------------|----------------------|-----------------|-----------------|
| Study day/period  | Grade                | n/N (%)              | n/N (%)              | n/N (%)              | n/N (%)         | n/N (%)         |
| Post first dose through follow-up | 0 | 0/6 (0.0) | 1/6 (16.7) | 0/6 (0.0) | 0/6 (0.0) | 1/12 (8.3) | 0/12 (0.0) |
|                   | 0.5                  | 0/6 (0.0)            | 4/6 (66.7)          | 1/6 (16.7)          | 0/6 (0.0)      | 4/12 (33.3)     | 1/12 (8.3)     |
|                   | 1                    | 5/6 (83.3)           | 0/6 (0.0)           | 5/6 (83.3)          | 4/6 (66.7)     | 5/12 (41.7)     | 9/12 (75.0)    |
|                   | 1.5                  | 0/6 (0.0)            | 0/6 (0.0)           | 0/6 (0.0)           | 0/6 (0.0)      | 0/12 (0.0)      | 0/12 (0.0)     |
|                   | 2                    | 1/6 (16.7)           | 1/6 (16.7)          | 0/6 (0.0)           | 1/6 (16.7)     | 2/12 (16.7)     | 1/12 (8.3)     |
|                   | 2.5                  | 0/6 (0.0)            | 0/6 (0.0)           | 0/6 (0.0)           | 0/6 (0.0)      | 0/12 (0.0)      | 0/12 (0.0)     |
|                   | 3                    | 0/6 (0.0)            | 0/6 (0.0)           | 0/6 (0.0)           | 0/6 (0.0)      | 0/12 (0.0)      | 1/12 (8.3)     |

Sequence 1, (AM→PM); sequence 2, (PM→AM); AM, once daily morning; PM, once daily evening.

Score interpretation: ≥0 or 0.5, no hyperaemia; 1 or 1.5, mild hyperaemia; 2 or 2.5, moderate hyperaemia; 3, severe hyperaemia; when two eyes in the same subject had different results, the worse (more severe grade) score was used.

**Table 3: Adverse events**

| System organ class | Pooled AM (n=12) | Pooled PM (n=12) | All (n=12) |
|--------------------|-----------------|-----------------|-----------|
| preferred term     | n (%)           | n (%)           | n/%       |
| Number of AEs/number of subjects (% of subjects with $\geq$ 1 AE) | 2/2 (16.7) | 11/6 (50.0) | 13/7 (58.3) |
| Dry eye disorders | 0 (0.0) | 6 (50.0) | 6 (50.0) |
| Dry eye            | 0 (0.0) | 0 (0.0)  | 3 (25.0)  |
| Eye pruritus       | 0 (0.0) | 0 (0.0)  | 1 (8.3)   |
| Ocular hyperaemia  | 0 (0.0) | 6 (50.0) | 6 (50.0) |
| Gastrointestinal disorders | 1 (8.3) | 0 (0.0) | 1 (8.3) |
| Dyspepsia          | 1 (8.3) | 0 (0.0)  | 1 (8.3)   |
| Skin and subcutaneous tissue disorders | 1 (8.3) | 1 (8.3) | 2 (16.7) |
| Dermatitis contact | 0 (0.0) | 1 (8.3)  | 1 (8.3)   |
| Urticaria          | 1 (8.3) | 0 (0.0)  | 1 (8.3)   |

AE, adverse event.
on day 15, mean IOP was 16.4 mm Hg and reduction relative to baseline at 08:00 (12 h after dose) was $-7.5 \text{ mm Hg}$ ($-31.0\%$). At 24 h post dose at 20:00, mean observed IOP was 14.6 mm Hg and reduction relative to baseline was $-4.5 \text{ mm Hg}$ ($-22.5\%$). After 14 days of dosing, the actual values for IOP were 16.8 and 16.4 mm Hg at 08:00 h on day 14 in the AM dosing group and day 15 in the PM dosing group and 14.0 and 14.3 mm Hg for AM and PM dosing, respectively, at 22:00 h.

Although pooled data (table 4) indicated that a greater number of subjects responded to treatment with IOP lowering to levels $\leq 18 \text{ mm Hg}$ during the 14-day AM treatment intervals (10/12 subjects, 83.3\%) versus PM treatment intervals (8/12 subjects, 66.7\%), greater numbers of subjects in the pooled 14-day PM treatment intervals than AM treatment intervals met IOP lowering targets of $\leq 17 \text{ mm Hg}$ (8/12 subjects, 66.7\%, vs 5/12, 41.7\%) and $\leq 16 \text{ mm Hg}$ (7/12 subjects, 58.3\%, vs 3/12, 25\%).

Pharmacokinetics

Plasma concentrations of ONO-9054 were below the limit of quantification in all subjects. ONO-AG-367 was detectable (see online supplementary figures S2 and S3). Maximum plasma concentrations on day 1 were achieved after approximately 10 min for each dosing schedule (mean T$_{\text{max}}=0.17 \text{ h}$). Maximum plasma concentrations were slightly higher for AM than for PM dosing (mean C$_{\text{max}} 23.4 \text{ pg/mL}$ vs 20.4 pg/mL) and T$_{1/2}$ was slightly shorter (mean T$_{1/2} 0.84 \text{ h}$ vs 0.96 h).

DISCUSSION

ONO-9054 was well tolerated in both dosing groups, although there were some indications that ONO-9054 might have been better tolerated in the AM dosing sequence. Most adverse events occurred in the PM dosing group, although they were all considered mild. Notably, there were no ocular adverse events in the AM dosing group; ocular adverse events were only reported in the PM dosing group. Dry eye has been reported for several pharmacological treatments of OAG, and visual function is known to be significantly better in the mornings than in the evenings due to regeneration of the tear film while the eyes are closed. As such, the dryness reported here is likely to originate from the active compound potentiating dryness accumulating throughout the day. There is a possibility that the frequency of adverse events reported in this study may have been augmented because of the twice-daily dosing schedule used to implement the crossover design. Subjects received vehicle twice daily—one as placebo and once carrying ONO-9054. Ocular irritation may have been exacerbated as this administration was double what subjects would usually experience on a once-daily schedule.

### Table 4

| Pressure target (mm Hg) | Pooled AM 25 h post AM dose n/N (%) | Pooled PM 25 h post PM dose n/N (%) |
|------------------------|-------------------------------------|-------------------------------------|
| $>18$                  | 2/12 (16.7)                         | 4/12 (33.3)                         |
| $\leq 18$              | 10/12 (83.3)                        | 8/12 (66.7)                         |
| $\leq 17$              | 5/12 (41.7)                         | 8/12 (66.7)                         |
| $\leq 16$              | 3/12 (25.0)                         | 7/12 (58.3)                         |
| $\leq 15$              | 3/12 (25.0)                         | 5/12 (41.7)                         |
| $\leq 14$              | 1/12 (8.3)                          | 3/12 (25.0)                         |
| $\leq 13$              | 0/12 (0)                            | 1/12 (8.3)                          |

Figure 1 Observed intraocular pressure (IOP) values following morning (AM) and evening (PM) dosing of ONO-9054. IOP decreased from baseline in both cohorts, and this was maintained throughout the dosing period. Data from both dosing sequences were pooled to produce data for AM (♦) and PM (▴) dosing.
The most common local side effect of FP receptor agonists is mild to moderate ocular hyperaemia: rates of 5–20% with latanoprost, 35–50% with travoprost and 15–55% with bimatoprost have been reported. Given the small population of the current study, comparisons with previously published rates would be unreliable; two (17%) subjects in both the AM and PM groups experienced hyperaemia and the incidence of ocular hyperaemia was similar in both dosing schedules. There were no meaningful pharmacokinetic differences between AM and PM dosing.

IOP displays diurnal variation. Given that IOP is often highest when measured on awakening, there is a possibility that the most serious IOP-related damage occurs early in the morning, before patients have the opportunity to instil medication into their eyes. Administration of ONO-9054 led to reductions in IOP over the 14-day dosing period irrespective of whether it was administered AM or PM. ONO-9054 produced substantial IOP lowering as early as day 2 of the study. The peak and trough IOP values were observed with either AM or PM dosing.

After 14 days, ONO-9054 achieved IOP reductions comparable with those of bimatoprost, latanoprost and travoprost reported after 1 month. For exploratory purposes, this study which was designed to provide a gross comparison of AM and PM dosing included only 12 subjects. A crossover study design has benefits compared with a parallel design in reducing variability by using within-subject comparisons. Typically, such a design would be expected to have a greater power than the equivalent parallel design; however, no sample size assumptions were made and the effects of AM and PM administration of ONO-9054 were not compared statistically. Data for maximum trough IOP values were observed with either AM or PM dosing.

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Competing interests AF, TO and CLW are employees of ONO Pharmaceuticals Co. CR-R, DTR and AW conducted this work as paid employees of ONO Pharma USA, MSB, CQ and IA acted as consultants to Ono and were remunerated for their contributions.

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