Effect of 15-Keto Latanoprost on Intraocular Pressure and Aqueous Humor Dynamics in Monkey Eyes

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PURPOSE. To compare the ocular hypotensive effects of 15-keto latanoprost (KL) with the commercial preparation of latanoprost (Xalatan; Pfizer, New York, NY) in monkey eyes with laser-induced unilateral glaucoma and to evaluate the effects of topical 0.005% KL on aqueous humor dynamics in normal monkey eyes.

METHODS. Intraocular pressure (IOP) was measured hourly for 6 hours beginning at 9:30 AM on day 1 (untreated baseline); day 2 (vehicle only); and treatment days 1, 3, and 5 (topical, 30 µL of study drug) in the glaucomatous eyes of four to eight monkeys with unilateral laser-induced glaucoma. KL concentrations of 0.0001%, 0.001%, and 0.01% and latanoprost at 0.005% were studied separately, with a minimum washout period of 2 weeks between studies. Tonographic outflow facility (C) and fluorophotometric aqueous humor flow rates (F) were measured in nine normal monkeys before and after a single topical dose of 0.005% KL in one eye, with a vehicle-only control in the fellow eye.

RESULTS. When applied once daily to glaucomatous monkey eyes, all three concentrations of KL and a 0.005% concentration of latanoprost produced significant (P < 0.05) reductions in IOP, with the maximum reduction on treatment day 5, regardless of the drug or concentration studied. The maximum reduction (P < 0.001) from vehicle-only baseline IOP was (mean ± SEM) 3.0 ± 0.5 mm Hg (9%) for 0.0001% KL, 7.6 ± 0.6 mm Hg (23%) for 0.001% KL, 6.5 ± 0.4 mm Hg (18%) for 0.01% KL, and 6.6 ± 0.6 mm Hg (20%) for 0.005% latanoprost. After application of a single dose of 0.005% KL in nine normal monkey eyes, neither C nor F was altered (P > 0.80) when compared with untreated baseline values or vehicle-treated control eyes.

CONCLUSIONS. The reduction in IOP produced by 0.001% KL was equivalent to, and at some measured time points, greater than the effect produced by 0.005% latanoprost. The IOP reduction by KL in normal monkeys appeared to have no effect on aqueous humor production or tonographic outflow facility and may thus indicate a drug-induced increase in uveoscleral outflow. (Invest Ophthalmol Vis Sci. 2007;48:4143–4147) DOI:10.1167/iovs.07-0035

The clinically available prostaglandin analogs latanoprost, bimatoprost, travoprost,1 and isopropyl unoprostone2 are all isopropyl ester prodrugs and effective ocular hypotensive agents.

The majority of receptor-binding studies indicate that the free acid metabolites of these compounds are agonists at the prostaglandin FP receptor, but with widely differing affinities.3–8 These drugs lower intraocular pressure (IOP) in part by enhancing uveoscleral outflow,9–13 although the precise mechanism by which this occurs is not known.9–12 However, recent studies in mice lacking individual PG receptor subtypes indicate that the ocular hypotensive response of these PG drugs may actually be mediated by EP3 receptors activated by endogenously produced PGs resulting from drug stimulation of FP receptors.15,16 Some studies have also shown an increase in total outflow facility after treatment with isopropyl unoprostone,17 and to some extent also with latanoprost11,12 or bimatoprost,18 in addition to their increasing uveoscleral outflow.

Three of the four clinically used PG analogs have a hydroxyl group on position 15, which is the site of potential metabolic conversion into a 15-keto analog. The exception is unoprostone, which is an analog of 15-keto PGF2α, FP. We wanted to determine whether metabolic oxidation of the 15-hydroxyl function of ocular hypotensive PGF2α, FP analogs produces intrinsically less active PG analogs, which is commonly believed to be the case. Therefore, the present study was designed to evaluate the ocular hypotensive effect of 15-keto latanoprost (KL) in three concentrations in glaucomatous monkey eyes compared with its clinically approved 15-hydroxy analog, Xalatan (0.005% latanoprost; Pfizer, New York, NY). The mechanism by which KL alters aqueous humor dynamics was also evaluated, by using the 0.005% concentration in normal monkey eyes.

MATERIALS AND METHODS

Animals

Twenty-one adult female cynomolgus monkeys, weighing 3 to 6 kg, were used in the studies. Twelve monkeys, in which glaucoma had been unilaterally induced by repeated diode laser photocoagulation of the mid trabecular meshwork,19 were used to evaluate the ocular hypotensive effects of 0.0001%, 0.001%, and 0.01% KL and 0.005% latanoprost. In a separate experiment, nine normal monkeys without glaucoma were used to evaluate the effect of 0.005% KL on aqueous humor dynamics. All experimental studies complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Institutional Animal Care and Utilization Committee of Mount Sinai School of Medicine.

Preparation and Instillation of Testing Compounds

15-Keto latanoprost was obtained from two sources, termed KL-A and KL-B, and were tested at different times on different sets of monkeys. KL-A (from R-Tech Ueno, Tokyo, Japan) was used in most of the experiments. It was prepared synthetically and had analytical purity of 99.6% (batch 4, by HPLC). Solutions of 0.0001%, 0.001%, and 0.01% used for topical treatments were made up in the ophthalmic vehicle contained in Xalatan (isotonic saline containing Na EDTA and polysorbate 80, preserved with benzalkonium chloride; Pfizer) and were separately assayed (HPLC) for KL purity (95.1%, 94.2%, and 93.3%, respectively). The KL-B sample was obtained from Cayman Chemical.
Comparison of Various Doses of KL and 0.005% Latanoprost on IOP

The hypotensive response of the two drug samples, KL-A and -B, were independently compared (at different times and in different sets of glaucomatous monkey eyes) at the 0.001% single-dose level. Figure 1 indicates that KL from both sources

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<th>Table 1. Comparison of IOP Effects of Once-Daily Administration of KL-A or Latanoprost for 5 Days</th>
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Data are mean IOP (mm Hg) ± SEM. Each group consisted of 8 monkeys with unilateral laser-induced glaucoma. Number in parenthesis is the percent reduction of IOP compared to vehicle-only treatment. Trough (0 hr) is the IOP immediately before drug treatment on that day.

* Significant IOP reduction compared to vehicle-only treatment.
† Bonferroni \(t\) test, \(P < 0.001\).
‡ Bonferroni \(t\) test, \(P < 0.005\).
mined before treatment on days 3 and 5; Table 1, Fig. 2). The maximum reduction in IOP from vehicle-only baseline for both concentrations occurred 2 hours after each morning dose.

Treatment with 0.005% latanoprost produced a significant ($P < 0.001$) reduction in IOP from vehicle treatment levels at 1 to 5 hours after the first dose on day 1. A significant ($P < 0.02$) ocular hypotensive effect was maintained for a minimum of 24 hours after the second dose. The peak mean reduction of IOP from vehicle treatment levels measured 4.8 ± 0.5 mm Hg (14%) on day 1, 6.0 ± 0.3 mm Hg (18%) on day 3, and 6.6 ± 0.6 mm Hg (20%) on day 5 (Table 1, Fig. 3).

For all three concentrations of KL-A and for 0.005% latanoprost, the maximum reductions in IOP were increased on day 5 after repeated doses. When compared with 0.0001% KL-A, treatment with 0.001% KL-A produced a greater magnitude (23% vs. 9%; $P < 0.005$) and longer duration (24 hours vs. 4 hours; Fig. 2) of IOP reduction. Increasing the concentration to 0.01% did not further increase the magnitude of IOP reduction on days 1 and 3 ($P > 0.90$) but produced a slightly smaller magnitude of IOP reduction between concentrations (two-tailed paired $t$ test; *$P < 0.01$, *$0.001$ vs. 0.01%, **$0.001$ vs. 0.01%). Arrow: drug treatment immediately after time 0 measurement.

FIGURE 2. Comparison of the mean change in IOP in a group of eight glaucomatous monkey eyes after once-daily administration of 0.0001%, 0.001%, or 0.01% KL-A for 5 days. Data show the mean change in IOP from vehicle-only baseline. Asterisks indicate significant difference in magnitude of IOP reduction between concentrations (two-tailed paired $t$ test; $P < 0.01$, *0.0001% vs. 0.001%, **0.001% vs. 0.01%). Arrow: drug treatment immediately after time 0 measurement.

was probably equivalent: The peak response—a 15% to 20% reduction of IOP—occurred at 2 to 3 hours, and the response lasted for at least 6 hours.

In the multiple-dose experiments with KL-A the mean baseline and vehicle-only treated IOPs of the four treatment groups were not significantly different ($P > 0.90$, Table 1). All three concentrations of KL-A tested, (0.0001%, 0.001%, and 0.01%), and 0.005% latanoprost produced significant ($P < 0.001$) reductions from untreated baseline and vehicle-only IOP levels. Once-daily administration of 0.0001% KL-A for 5 days significantly ($P < 0.05$) reduced IOP at 2 hours after the first dose, and from 1 to 4 hours after the third and the fifth doses. Compared with vehicle-only baseline, the maximum reductions in IOP 2 hours after each morning dose for the three concentrations of KL-A are shown in Table 1.

Both 0.001% and 0.01% KL-A significantly ($P < 0.005$) reduced IOP for at least 6 hours after the first dose on day 1 and for at least 24 hours after the second and fourth doses (determined before treatment on days 3 and 5; Table 1, Fig. 2). The maximum reduction in IOP from vehicle-only baseline for both concentrations occurred 2 hours after each morning dose.

FIGURE 3. Comparison of the mean change in IOP in eight glaucomatous monkey eyes after once-daily administration of either 0.001% KL-A or 0.005% latanoprost for 5 days. Values are the mean change in IOP from vehicle-only baseline. Asterisks indicate significant difference in magnitude of IOP reduction between 0.001% KL-A and 0.005% latanoprost (two-tailed paired $t$ test; *$P < 0.05$, **$P < 0.005$). Arrow: drug treatment immediately after time 0 measurement.
reduction in IOP on day 5 at 0 (7% vs. 12%; \( P < 0.01 \)) and 4 (14% vs. 18%; \( P < 0.005 \)) hours.

Compared with 0.005% latanoprost, 0.001% KL-A produced a slightly longer duration of IOP reduction (6 hours vs. 5 hours) after the first dose on treatment day 1 and a greater (\( P < 0.05 \)) IOP reduction at 4 and 6 hours on day 1 and at 0 and 4 hours on day 5 (Fig. 3).

**Effect of KL-A on Aqueous Humor Dynamics**

Two hours after unilateral application of 0.005% KL-A to nine normal monkey eyes, outflow facility was unchanged compared with both vehicle-treated control eyes (\( P > 0.70 \)) and baseline values (\( P > 0.20 \)). IOP was significantly reduced (\( P < 0.01 \)) at 2 hours in the drug-treated eyes when measured tonographically (Table 2). For 4 hours after the administration of a single dose of 0.005% KL-A, aqueous humor flow rates were not altered compared with those in either vehicle-treated control eyes (\( P > 0.05 \)) or baseline values (\( P > 0.80 \); Table 2).

**DISCUSSION**

Latanoprost and related PGF\(_{2\alpha}\) isopropyl ester analogs are effective ocular hypotensive agents for the treatment of patients with elevated IOP. Oxidation of the C-15 hydroxyl group by 15-hydroxy prostaglandin dehydrogenase subsequent to isopropyl ester hydrolysis introduces a 15-keto group. This metabolite of latanoprost is produced by the NADP\(^+\)-dependent 15-PG dehydrogenase enzyme shown to be highly expressed in the monkey eye. The present comparison study on 15-keto latanoprost in a multiple-dose regimen in glaucomatous monkey eyes is the first to show a highly significant and potent ocular hypotensive effect of a 15-keto PGF\(_{2\alpha}\) analog that is equivalent to or even exceeds the response of its otherwise structurally identical 15-hydroxy PGF\(_{2\alpha}\) analog (latanoprost) at the same dose level.

Our results showed that once-daily administration of KL at concentrations as low as 0.0001% lowers IOP in glaucomatous monkey eyes. All three concentrations of KL tested and 0.005% latanoprost produced a sustained reduction in IOP for 5 days with once-daily doses. The 0.001% concentration of KL produced the greatest magnitude and longest duration of IOP reduction, whereas increasing the concentration to 0.01% caused a similar or lesser response, indicating that KL in a concentration of 0.001% is near or at the top of the dose-response curve.

In a comparison study, we showed that treatment with 0.001% KL produced an equivalent and, at some measured time points, a slightly greater reduction in IOP when compared with 0.005% latanoprost. Thus, KL at 0.001% appeared somewhat more potent (approximately fivefold by dose ratio) than latanoprost in the 0.001% to 0.005% dose range. To confirm the effect on IOP, we used KL from two different sources, prepared in the same vehicle but tested in separate groups of glaucomatous monkey eyes. The result indicated that the effect on IOP of KL-A and -B at the 0.001% dose was equivalent, with similar (\( P > 0.05 \)) maximum IOP reductions of 15% to 20%.

To try to determine the mechanism of the KL response, we investigated the effect of 0.005% KL on aqueous humor dynamics in normal monkey eyes. Neither tonographic outflow facility nor aqueous humor flow rates were significantly altered in normal monkey eyes treated with 0.005% KL. Assuming that episcleral venous pressure is unchanged and pseudofacility is ignored, the primary mechanism through which KL lowers IOP appeared to be by an increase in uveoscleral outflow.

Similar to KL, the ocular hypotensive drug isopropyl unoprostone is a 15-keto PGF\(_{2\alpha}\) analog. It has been directly compared with latanoprost\(^{21}\) in the same monkey protocols as were used in the present study, with similar results. The agonist activity of isopropyl unoprostone at FP receptors is reportedly the weakest among the ocular hypotensive PG drugs,\(^{6}\) and in one study no FP receptor affinity was found.\(^{3}\) However, it has effects on ion channels and cellular Ca fluxes\(^{22}\) that may not be FP receptor linked. These include activation of Ca-activated potassium channels (maxi-K\(^+\) channel)\(^{23,24}\) and blockade of Ca-release-activated Ca flux in trabecular meshwork cells.\(^{25}\) Thus, it is possible that 15,14-dihydro-15-keto PGF\(_{2\alpha}\) analogs, such as isopropyl unoprostone and KL, lower IOP by different receptor mechanisms than does latanoprost.

In conclusion, 15-keto prostaglandin analogs that may be endogenously produced from an administered 15-hydroxy analog have the potential to contribute to the ocular hypotensive response and independently may have potential for the direct treatment of glaucoma.

**References**


