

Aqueous humor penetration of topical bimatoprost 0.01% and bimatoprost 0.03% in rabbits

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Purpose: The purpose of this study was to compare the aqueous humor concentrations of bimatoprost acid after topical instillation in rabbits of bimatoprost ophthalmic solution 0.01% and bimatoprost ophthalmic solution 0.03%, two commercially available intraocular pressure-lowering medications.

Methods: Male Dutch Belted rabbits were divided into two treatment groups (four rabbits/eight eyes per group): bimatoprost 0.01% and bimatoprost 0.03%. Thirty microliters (μL) of study medication was topically instilled into both eyes of each animal. Thirty minutes and 90 minutes after instillation, aqueous humor samples were collected. These samples were analyzed by reverse-phase high-performance liquid chromatography for bimatoprost acid concentration.

Results: Following a single topical ocular instillation, the bimatoprost 0.01% formulation had a lower mean aqueous humor concentration of bimatoprost acid than the bimatoprost 0.03% formulation at both 30 minutes (11.5 ± 2.1 ng/mL versus 37.8 ± 28.8 ng/mL; $P = 0.17$) and 90 minutes (20.8 ± 5.7 ng/mL versus 45.8 ± 14.3 ng/mL; $P = 0.03$) after topical instillation.

Conclusions: Topical ocular instillation of bimatoprost 0.01% produced significantly lower bimatoprost acid concentration in the aqueous humor of rabbits than bimatoprost 0.03%, despite the 4-fold increase of benzalkonium chloride contained in bimatoprost 0.01%.

Keywords: aqueous humor, benzalkonium chloride, bimatoprost, pharmacokinetics, preclinical, prostaglandin analog

Introduction

Patients with glaucoma, the second leading cause of vision loss worldwide,¹ are often treated with prostaglandin analogs to reduce elevated intraocular pressure (IOP). Bimatoprost 0.03% (Lumigan[®], Allergan Inc, Irvine, CA) is one such prostaglandin analog that decreases IOP by promoting uveoscleral aqueous outflow via activation of the prostaglandin F receptor.² Bimatoprost is a prodrug that is hydrolyzed by esterases to release its active moiety, bimatoprost acid, into the aqueous humor.² Although bimatoprost is efficacious in reducing elevated IOP in patients with open-angle glaucoma or ocular hypertension,³ it is associated with a number of side effects, the most frequent of which is conjunctival hyperemia, affecting up to 45% of patients and accounting for discontinuation of therapy in 3% of patients.⁴

A new formulation of bimatoprost containing a lower concentration of drug (Lumigan 0.01%, Allergan Inc) has been developed in an attempt to improve the safety profile of this product.⁵ Bimatoprost 0.01% also contains a 4-fold greater concentration of the commonly used ophthalmic preservative, benzalkonium chloride (BAK), than bimatoprost 0.03% (0.02% BAK versus 0.005% BAK). In addition to its function as a

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preservative, in vitro evidence suggests that BAK may facilitate topical ocular drug delivery by increasing transcorneal drug penetration of some medications,⁶ which another study demonstrated can occur in a dose-dependent manner.⁷ The goal of the current study was to determine whether bimatoprost ophthalmic solution 0.01% with 0.02% BAK improves ocular penetration relative to bimatoprost ophthalmic solution 0.03% with 0.005% BAK by comparing the aqueous humor concentrations of bimatoprost acid, the active metabolite of bimatoprost, after topical instillation of both commercial formulations of bimatoprost in rabbits.

Methods

Animals

Eight healthy male Dutch Belted rabbits weighing approximately 2 kg were used. Animals were treated in accordance with the Association for Research in Vision and Ophthalmology statement for Use of Animals in Ophthalmic and Vision Research. The study was conducted at an independent contract laboratory (PharmOptima, Portage, MI).

Study design

Rabbits (eight eyes per group) were treated with one of two commercially available bimatoprost formulations: bimatoprost ophthalmic solution 0.01% (Lumigan 0.01%, Allergan Inc, Ontario, Canada) or bimatoprost ophthalmic solution 0.03% (Lumigan 0.03%, Allergan Inc, Irvine, CA). Thirty microliters of the study medication was instilled in both eyes of each animal. For each group, 50 μ L aqueous humor samples were collected from each eye using a 28-gauge, 0.5-inch needle at 30 minutes or 90 minutes after study medication instillation, for a total of four samples per time point. Each sample was analyzed for bimatoprost acid concentration.

Sample preparation

Each aqueous humor sample was mixed with 50 μ L methanol and 100 μ L acetonitrile containing reserpine (internal standard) solution at 2.0 μ g/mL. All samples were vortexed for 30 seconds, followed by centrifugation at 14,000 rpm and at 4°C for 10 minutes. The supernatants were decanted into autosampler vials and stored at -20°C until time of analysis by high-performance liquid chromatography (HPLC).

High-performance liquid chromatography

Aqueous humor concentrations of bimatoprost acid were determined by an independent laboratory using a validated HPLC method. Chromatography was performed on a Hypersil

Gold, 50 \times 2.1 mm, 5 μ m with precolumn filter (Thermo Scientific, Rockford, IL). The mobile phase consisted of firstly 0.1% formic acid in water and secondly 0.1% formic acid in methanol. Analysis of 10 μ L samples was performed on a TSQ Quantum Access (Thermo Scientific). The lower limit of quantitation for bimatoprost acid was 1.00 ng/mL.

Results

As shown in Figure 1, the mean aqueous humor concentration of bimatoprost acid was lower in the bimatoprost 0.01% group than in the bimatoprost 0.03% group after 30 minutes (11.5 \pm 2.1 ng/mL versus 37.8 \pm 28.8 ng/mL; $P = 0.17$) and 90 minutes (20.8 \pm 5.7 ng/mL versus 45.8 \pm 14.3 ng/mL; $P = 0.03$) following topical administration. The mean aqueous humor concentration of bimatoprost acid at 30 minutes and 90 minutes postdose was approximately 3.3-fold and 2.2-fold lower, respectively, in the bimatoprost 0.01% group than in the bimatoprost 0.03% group.

Discussion

Although the original formulation of bimatoprost ophthalmic solution has a favorable IOP-lowering efficacy,⁸⁻¹⁰ it produces conjunctival hyperemia in nearly half of patients.⁴ The newer formulation of bimatoprost was designed to improve upon the tolerability of topical bimatoprost by lowering the drug concentration from 0.03% to 0.01%. The amount of BAK was increased 4-fold (from 0.005% BAK to 0.02% BAK) in the revised bimatoprost 0.01% formulation, in an attempt to compensate for the 67% reduction in bimatoprost concentration and the expected loss of ocular bioavailability of the revised formulation.⁵ BAK has been shown to improve corneal

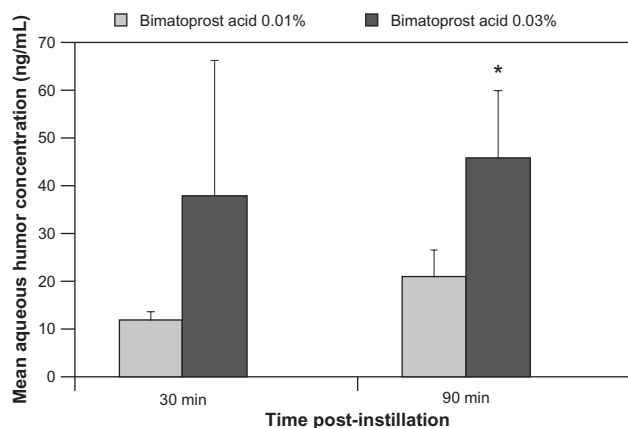


Figure 1 Mean aqueous humor concentrations ($n = 4$ each) of bimatoprost acid after instillation of bimatoprost 0.01% or bimatoprost 0.03%.

Notes: * $P = 0.03$ bimatoprost acid 0.01% vs bimatoprost acid 0.03%. Student's t -test. Error bars denote standard deviations.

penetration in some animal models,^{6,11} possibly through the loss of tight junctions in the corneal epithelium.^{11,12} However, in the current in vivo rabbit pharmacokinetic study of these two commercially available bimatoprost products, the newer 0.01% formulation demonstrated a lower mean aqueous humor concentration of bimatoprost acid than the original 0.03% formulation after a single topical ocular dose; this reduction in concentration was approaching statistical significance at 30 minutes and was statistically significant at 90 minutes post-dose. The results of this pharmacokinetic study show that, despite the 4-fold increase in BAK concentration in the revised bimatoprost 0.01% formulation, it has significantly less ocular bioavailability than the bimatoprost 0.03% formulation. Thus, the bimatoprost 0.01% group did not demonstrate improved corneal penetration when compared with the bimatoprost 0.03% group, up to 90 minutes after topical ocular administration, despite the increased BAK concentration in the bimatoprost 0.01% formulation.

The decreased aqueous humor concentration of bimatoprost acid observed with the lower concentration may translate into less drug reaching the target site of action, which could compromise the IOP-lowering effect of the revised bimatoprost 0.01% formulation. The only clinical study published that compares these two formulations showed that the bimatoprost 0.03% group had a numerically greater mean decrease in IOP (from baseline) than the bimatoprost 0.01% group at nearly every time point reported over the 12 months of the study, but these differences were within the 1.5 mmHg limit that designated a clinically relevant difference.⁵

The revised bimatoprost formulation may have an increased risk of ocular toxicity, particularly to the ocular surface. Numerous preclinical studies have established not only that BAK causes both corneal and conjunctival toxicity, but also that this BAK toxicity is dose-dependent.^{13–16} Furthermore, clinical studies of glaucoma patients have reported increased ocular toxicity with IOP-lowering medications containing BAK.^{17,18}

Because aqueous humor concentrations in the current study were measured only to 90 minutes postdose, no conclusions can be drawn regarding penetration that might have occurred after this time. However, within the parameters of the study design, the current study demonstrated that the increased BAK concentration in bimatoprost 0.01% did not adequately compensate for its 67% decrease in drug concentration, resulting in lower aqueous humor drug

concentrations than bimatoprost 0.03%. Due to the potential differences between rabbits and humans with respect to both corneal penetration of bimatoprost and response to BAK, the corneal penetration of the two commercial formulations of bimatoprost investigated in this study should be evaluated in a clinical trial. Furthermore, the long-term efficacy and safety of this new bimatoprost formulation can only be determined in a randomized clinical trial of adequate duration.

Disclosure

Medical writing assistance for this paper, provided by Jennifer Klem, PhD, was funded by Alcon Laboratories, Inc.

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