In vivo comparative study of ocular vasodilation, a relative indicator of hyperemia, in guinea pigs following treatment with bimatoprost ophthalmic solutions 0.01% and 0.03%

Abayomi B Ogundele
David Earnest
Marsha A McLaughlin
Alcon Research, Limited, Fort Worth, TX, USA

Objective: The objective of this in vivo study was to compare the incidence of vasodilation in guinea pigs following topical administration of bimatoprost ophthalmic solutions 0.01% and 0.03%.

Methods: The study comprised 20 guinea pigs assigned to 2 treatment groups (10 per treatment group) to receive either bimatoprost 0.01% or bimatoprost 0.03%. Animals were hand-held under 2.75 × magnification to score ocular vasodilation (a measure of hyperemia), using a scoring system developed at Alcon Research, Ltd. Following baseline ocular scoring, each animal received a 30 µL dose to the left eye of either bimatoprost 0.01% (3 µg) or bimatoprost 0.03% (9 µg). Vasodilation was again scored at 1, 2, 3, 4, 5 and 6 hours after dosing. Incidence of vasodilation was calculated as the percent of total eyes in each 2-hour time interval with scores ≥2.

Results: The incidence of vasodilation was higher in the bimatoprost 0.01% treatment group (range, 45.0% to 60.0%) than the bimatoprost 0.03% treatment group (range, 30.0% to 52.2%) at all post-dosing time points.

Conclusion: The 2 bimatoprost formulations elicited ocular vasodilation of long duration (>6 hours) in the guinea pig model, with the bimatoprost 0.01% treatment group showing a higher incidence of ocular vasodilation than the bimatoprost 0.03% treatment group. Further clinical studies would be needed to determine whether the higher incidence of vasodilation may also be attributed to the increased BAK concentration in the bimatoprost 0.01% formulation.

Keywords: bitamoprost, ocular vasodilation, hyperemia

Introduction
The goal of therapy for patients with glaucoma is prevention of progressive optic nerve damage, which is achieved by lowering the intraocular pressure (IOP).1 The prostaglandin analogs (PGA) latanoprost ophthalmic solution 0.005% (Xalatan®, Pfizer, New York, NY, USA), travoprost ophthalmic solution 0.004% (TRAVATAN®, Alcon Laboratories, Inc., Fort Worth, TX, USA), and bimatoprost ophthalmic solution 0.03% (Lumigan®, Allergan, Inc., Irvine, CA, USA), have become the most prominent topical treatments for ocular hypertension and open-angle glaucoma due to their potency and efficacy at lowering IOP and their favorable safety profile.1,2

Bimatoprost is an ethyl amide pro-drug derivative of the potent but non-selective FP prostaglandin receptor agonist; 17-phenyl-trinor PGF2α.3,4 Studies conducted on laboratory animals have demonstrated the topical and systemic safety profiles of bimato-
However, several clinical trials demonstrated that the most common ocular side effect of bimatoprost in humans is ocular hyperemia. Bimatoprost 0.03% is preserved with 0.005% benzalkonium chloride (BAK). Bimatoprost 0.01% is a new formulation approved in some countries, containing the same active ingredient, but in lower concentration, and a 4-fold increase in the amount of BAK (0.02%).

Conjunctival hyperemia is a non-specific clinical term that implies vasodilation and the resultant increased blood supply to the conjunctival blood vessels. Ocular hyperemia is caused by pharmacologic and/or inflammatory mechanisms and is a very common side effect of IOP-lowering medications, with prostaglandin analogs being associated with the highest incidences of hyperemia. The rate of hyperemia of bimatoprost, as listed in the product labeling, ranges from 15% to 45%.

The objective of this study was to compare the incidence of vasodilation in guinea pigs following topical ocular administration of bimatoprost 0.01% and bimatoprost 0.03%.

Materials and methods

Animals

Twenty Hartley Outbred guinea pigs weighing 771 to 1078 g were obtained from Charles River Laboratories (Montreal, Quebec, Canada). Animals were divided into 2 groups, 10 animals per group, to receive either commercially available bimatoprost 0.01% (Lumigan 0.01%, Allergan Inc., Ontario, Canada) or bimatoprost 0.03% (Lumigan 0.03%, Allergan Inc., Irvine, CA, USA). Animals were housed individually and had unrestricted access to guinea pig chow and water.

Animals were handled and maintained according to the principles of the Declaration of Helsinki and in accordance with the Guiding Principles in the Care and Use of Animals. The study was approved by Alcon Research Ltd., Animal Study Review Board and followed the Association for Research in Vision and Ophthalmology (ARVO) guidelines for the use of animals.

Study design

Animals were hand-held under 2.75× magnification to score the magnitude of ocular vasodilation using a scoring system developed at Alcon Research, Ltd. Animals were selected for the study if the vasodilation score at baseline measurement was 0 (zero). After baseline scoring, all animals in each group received three 10 μL aliquots (approximately 3 minutes apart), of either bimatoprost 0.01% (3 μg) or bimatoprost 0.03% (9 μg) in the left eye only. Vasodilation was scored again using the same scoring system at 1, 2, 3, 4, 5, and 6 hours after dosing.

Alcon-developed hyperemia scoring system

Area of vasodilation

- 0 = Normal appearance of vessels at limbus and on rectus muscle
- 1 = Enlargement of vessels normally visible at limbus and on rectus muscle
- 2 = Branching of vessels at limbus, new vessels are visible
- 3 = New vessels visible in open bulbar conjunctival areas
- 4 = Diffuse redness in open bulbar conjunctival areas

0 and 1 are normal scores; 2, 3 and 4 represent adverse events.

Data calculation

Data were reported as percent incidence of vasodilation at 2-, 4-, and 6-hour intervals following dosing. Percent incidence is the total number of scores ≥2 divided by the number of observations per group. In this study with 10 treated eyes per group and time points at 1, 2, 3, 4, 5, 6 hours after dosing, the 2-hour interval had 20 observations per treatment group (cumulative observations of hours 1 and 2). The 4-hour and 6-hour intervals, had 40 and 60 observations, respectively (cumulative observation of hours 1 to 4 and hours 1 to 6, respectively). For example, if at the 4-hour interval there were 8 observations of vasodilation scores ≥2, then the percent incidence of vasodilation would be 20% (8/40 × 100).

Results

The incidence of vasodilation was higher in the bimatoprost 0.01% treatment group (range, 45.0% to 60.0%) than in the bimatoprost 0.03% treatment group (range, 30.0% to 52.2%) at all time points following dosing (Table 1).

Discussion

Glaucoma is an optic neuropathy characterized by increased IOP and progressive optic nerve damage. If left untreated, glaucoma can cause irreversible vision loss and is considered the second leading cause of blindness in the world. Management of glaucoma aims to reduce IOP using topical pharmacotherapy, such as prostaglandin analogs. Today, prostaglandin analogs are used as first-line therapy for glaucoma due to their efficacy at reducing IOP.
and their mild side effect profile, replacing the β-blockers for these indications.\textsuperscript{15}

Because glaucoma is a chronic condition, once topical therapy is initiated, many patients continue to use their IOP-lowering therapy for the rest of their lives, producing long-term exposure to the various excipients and preservatives used in these medications, which have been shown to cause corneal and conjunctival toxicity.\textsuperscript{8,16,17} Previous studies have shown that as many as 40% of glaucoma patients use more than one topical medication,\textsuperscript{18} and BAK is the most common preservative used in these preparations. Therapeutic regimens consisting of multiple BAK-containing medications can induce and/or exacerbate ocular toxicity, such as corneal epithelial cell dysfunction, altering the shape of the cornea and the consequential effect on accurate IOP measurement,\textsuperscript{16} and inflammatory and toxic effects on the conjunctiva.\textsuperscript{8,17} BAK has been shown to be responsible for at least part of the hyperemia associated with IOP-lowering therapy, as evidenced by a reduction of conjunctival hyperemia in patients who changed to a BAK-free prostaglandin analog after being treated with a BAK-preserved prostaglandin analog.\textsuperscript{19} Therefore, a BAK-free prostaglandin analog would decrease the load of this preservative on the ocular surface for patients using multiple topical medications.\textsuperscript{20}

The incidence of hyperemia caused by IOP-lowering medications is highly variable, depending on the methods and analyses of the various clinical trials. The rate of hyperemia of bimatoprost ranges from 15% to 45%.\textsuperscript{10} This side effect is theorized to be due to the vasodilation of the conjunctival vessels due to exposure to nitric oxide (NO), resulting from prostaglandin analog-induced overproduction of NO synthase,\textsuperscript{21} but not due to conjunctival inflammation.\textsuperscript{7,8,22} Additionally, Guenoun et al reported that although latanoprost contains higher concentration of BAK than the bimatoprost 0.03% formulation, it caused less hyperemia than bimatoprost 0.03%, suggesting that hyperemia is not solely a direct consequence of BAK toxicity, but also can be attributed to the prostaglandin analog molecule.\textsuperscript{8}

In this study, guinea pigs exposed to bimatoprost 0.01% and bimatoprost 0.03% developed ocular vasodilation within 2 hours after dosing, which lasted for more than 6 hours. Exposure to bimatoprost 0.01% caused higher incidences of vasodilation than exposure to bimatoprost 0.03% at all calculated intervals after dosing. The use of the incidence at individual time points helps in demonstrating the onset and duration of vasodilation. This study demonstrated that the 2 formulations of bimatoprost induced vasodilation soon after instillation, as evidenced by 2-hour percent incidence of 45% and 30% for bimatoprost 0.01% and bimatoprost 0.03%, respectively, and that the vasodilation lasted for at least 6 hours after dosing, based on the 4- and 6-hour incidence data. It is important to know that despite the lower concentration of bimatoprost 0.01%, the incidence of vasodilation was higher in this treatment group than the bimatoprost 0.03% treatment group. This higher incidence of vasodilation in the bimatoprost 0.01% formulation may be attributed to the 4-fold increase in the amount of BAK in this formulation (0.02%), as compared to the bimatoprost 0.03% formulation (0.005%).

This in vivo animal study assessed vasodilation, a relative indicator of hyperemia, after a single exposure to bimatoprost. Therefore, the outcome of the application of this model to multiple doses of bimatoprost is unknown. For this reason, results of this in vivo study should be confirmed in clinical studies.

**Conclusion**

This study demonstrates that both bimatoprost 0.01% and bimatoprost 0.03% formulations elicit ocular vasodilation at early post-instillation time points that lasts for longer than 6 hours in the guinea pig model, with the bimatoprost 0.01% treatment group showing a higher incidence of vasodilation than the bimatoprost 0.03% treatment group. Further clinical studies would be needed to determine whether the higher incidence of vasodilation may also be attributed to the increased BAK concentration in the bimatoprost 0.01% formulation.

**Acknowledgments/Disclosures**

The study was conducted at Alcon Research, Ltd., Fort Worth, TX, USA. All authors are employees of Alcon Research, Ltd. Heba Costandy, MD, MS, of H H Consulting, Inc., provided

---

**Table 1** Percent incidence of ocular vasodilation following administration of two bimatoprost formulations in guinea pigs

| Formulation       | 2-hour percent incidence | 4-hour percent incidence | 6-hour percent incidence |
|-------------------|--------------------------|--------------------------|--------------------------|
| Bimatoprost 0.01% (3 μg) | 45.0                     | 60.0                     | 55.0                     |
| Bimatoprost 0.03% (9 μg) | 30.0                     | 52.2                     | 48.3                     |
editorial assistance in the preparation of this manuscript. Alcon Research, Ltd., provided financial support for this assistance.

References

1. Maier PC, Funk J, Schwarzer G, Antes G, Faěk-Ytter YT. Treatment of ocular hypertension and open angle glaucoma: meta-analysis of randomised controlled trials. BMJ. 2005;331(7509):134.

2. Noecker RS, Dirks MS, Choplin NT, Bernstein P, Batoosingh AL, White CP. A six-month randomized clinical trial comparing the intraocular pressure-lowering efficacy of bimatoprost and latanoprost in patients with ocular hypertension or glaucoma. *Am J Ophthalmol*. 2003;135(1):55–63.

3. Sharif NA, Kelly CR, Williams GW. Bimatoprost (Lumigan(R)) is an agonist at the cloned human ocular FP prostaglandin receptor: real-time FLIPR-based intracellular Ca(2+) mobilization studies. *Prostaglandins Leukot Essent Fatty Acids*. 2003;68(1):27–33.

4. Sharif NA, Williams GW, Kelly CR. Bimatoprost and its free acid are prostaglandin FP receptor agonists. *Eur J Pharmacol*. 2001;432(2–3):211–213.

5. Woodward DF, Phelps RL, Krauss AH, et al. Bimatoprost: a novel antiglaucoma agent. *Cardiovasc Drug Rev*. 2004;22(2):103–120.

6. Abelson MB, Mroz M, Rosner SA, Dirks MS, Hirabayashi D. Multicenter, open-label evaluation of hyperemia associated with use of bimatoprost in adults with open-angle glaucoma or ocular hypertension. *Adv Ther*. 2003;20(1):1–13.

7. Chen J, Dinh T, Woodward DF, et al. Bimatoprost mechanism of ocular surface hyperemia associated with topical therapy. *Cardiovasc Drug Rev*. 2005;23(3):231–246.

8. Guenoun JM, Baudouin C, Rat P, Pauly A, Warnet JM, Brignole-Baudouin F. In vitro study of inflammatory potential and toxicity profile of latanoprost, travoprost, and bimatoprost in conjunctiva-derived epithelial cells. *Invest Ophthalmol Vis Sci*. 2005;46(7):2444–2450.

9. Brandt JD, VanDenburgh AM, Chen K, Whitcup SM. Comparison of once- or twice-daily bimatoprost with twice-daily timolol in patients with elevated IOP: a 3-month clinical trial. *Ophthalmology*. 2001;108(6):1023–1031.

10. Medical Economics. *Physicians’ Desk Reference (PDR)* for ophthalmic medicine. Montvale, NJ: 2002.

11. Sharif NA, McLaughlin MA, Kelly CR. AL-34662: a potent, selective, and efficacious ocular hypotensive serotonin-2 receptor agonist. *J Ocul Pharmacol Ther*. 2007;23(1):1–13.

12. Sharif NA, McLaughlin MA, Kelly CR, et al. Preclinical pharmacology of AL-12182, a new ocular hypotensive 11-oxa prostaglandin analog. *J Ocul Pharmacol Ther*. 2006;22(5):291–309.

13. Weinreb RN, Khaw PT. Primary open-angle glaucoma. *Lancet*. 2004;363(9422):1711–1720.

14. Congdon N, O’Colmain B, Klaver CC, et al. Causes and prevalence of visual impairment among adults in the United States. *Arch Ophthalmol*. 2004;122(4):477–485.

15. Chew PT, Aung T, Aquino MV, Rojanapongpun P. Intraocular pressure-reducing effects and safety of latanoprost versus timolol in patients with chronic angle-closure glaucoma. *Ophthalmology*. 2004;111(3):427–434.

16. Cha SH, Lee JS, Oum BS, Kim CD. Corneal epithelial cellular dysfunction from benzalkonium chloride (BAC) in vitro. *Clin Experiment Ophthalmol*. 2004;32(2):180–184.

17. Pisella PJ, Debbasch C, Hamard P, et al. Conjunctival proinflammatory and proapoptotic effects of latanoprost and preserved and unpreserved timolol: an ex vivo and in vitro study. *Invest Ophthalmol Vis Sci*. 2004;45(5):1360–1368.

18. Kass MA, Heuer DK, Higginbotham EJ, et al. The Ocular Hypertension Treatment Study: a randomized trial determines that topical ocular hypotensive medication delays or prevents the onset of primary open-angle glaucoma. *Arch Ophthalmol*. 2002;120(6):701–713.

19. Henry JC, Peace JH, Stewart JA, Stewart WC. Efficacy, safety, and improved tolerability of travoprost BAK-free ophthalmic solution compared with prior prostaglandin therapy. *Clin Ophthalmol*. 2008;2(3):613–621.

20. Whitson JT, Cavanagh HD, Lakshman N, Petroll WM. Assessment of corneal epithelial integrity after acute exposure to ocular hypotensive agents preserved with and without benzalkonium chloride. *Adv Ther*. 2006;23(5):663–671.

21. Astin M, Stjernschutz J, Selen G. Role of nitric oxide in PGF2 alpha-induced ocular hyperemia. *Exp Eye Res*. 1994;59(4):401–407.

22. Leal BC, Medeiros FA, Medeiros FW, Santo RM, Susanna R Jr. Conjunctival hyperemia associated with bimatoprost use: a histopathologic study. *Am J Ophthalmol*. 2004;138(2):310–313.