BMJ open

Twenty-four-hour effects of bimatoprost 0.01% monotherapy on intraocular pressure and ocular perfusion pressure

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-001106
Article Type:	Research
Date Submitted by the Author:	02-Mar-2012
Complete List of Authors:	Tung, Jonathan; University of California, San Diego, Ophthalmology Tafreshi, Ali; University of California, San Diego, Ophthalmology Weinreb, Robert; University of California, San Diego, Ophthalmology Slight, J; University of California, San Diego, Ophthalmology Medeiros, Felipe; University of California, San Diego, Ophthalmology Liu, John; University of California, San Diego, Ophthalmology
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Dermatology
Keywords:	Anaesthesia in ophthalmology < ANAESTHETICS, Anaesthesia in otolaryngology < ANAESTHETICS, Paediatric intensive & critical care < ANAESTHETICS

Twenty-Four-Hour Effects of Bimatoprost 0.01% Monotherapy On Intraocular Pressure and Ocular Perfusion Pressure

Jonathan D. Tung, MD

Ali Tafreshi, BSc

Robert N. Weinreb, MD

J. Rigby Slight, MD

Felipe A. Medeiros, MD, PhD

John H.K. Liu, PhD

Hamilton Glaucoma Center and Department of Ophthalmology, University of California, San Diego, La Jolla, California, USA.

Space count for title: 111. Word count for abstract: 194. Word count for text: 2067.

Running head: 24-hour effects of bimatoprost 0.01%.

Tables and figures: 1 table and 2 figures.

Corresponding author: John H.K. Liu, PhD, University of California, San Diego, Department of Ophthalmology, La Jolla, CA 92093-0946, USA.

Phone: (858) 534-7056. Fax: (858) 534-7014. E-mail: joliu@ucsd.edu.

ABSTRACT

Aim: To investigate the 24-hour effects of bimatoprost 0.01% monotherapy on intraocular pressure (IOP) and ocular perfusion pressure (OPP) in a prospective, open-label experimental study.

Methods: Sixteen patients with diagnosed primary open-angle glaucoma (POAG) or ocular hypertension (ages, 49–77 years) were enrolled. Baseline data of 24-hour IOP in untreated patients were collected in a sleep laboratory. Measurements of IOP were taken using a pneumatonometer every 2 hours in the sitting and supine body positions during the 16-hour diurnal/wake period and in the supine position during the 8-hour nocturnal/sleep period. After baseline measurements were taken, patients were treated with bimatoprost 0.01% one time per day at bedtime for 4 weeks, then 24-hour IOP data were collected under the same laboratory conditions. Diurnal and nocturnal IOP and OPP means under bimatoprost 0.01% treatment were compared with baseline.

Results: The diurnal and nocturnal IOP means were significantly lower under the bimatoprost 0.01% treatment than baseline in both the sitting and supine positions. The diurnal and nocturnal OPP means were significantly higher under treatment than baseline in both the sitting and supine positions.

Conclusion: Bimatoprost 0.01% monotherapy significantly lowered IOP and increased OPP during the 24-hour period.

INTRODUCTION

Glaucoma is a progressive optic neuropathy characterized by pathologic loss of retinal ganglion cells and retinal nerve fiber layer with associated visual field loss. The lowering of intraocular pressure (IOP) is the only proven method to reduce the risk of glaucoma onset and slow disease progression.[1] Studies have also shown an association between primary open-angle glaucoma (POAG) and vascular factors, such as systemic hypertension, hypotension, vasospasm, atherosclerosis, and ocular blood flow.[1,2] While the association between systemic blood pressure and POAG is weak, ocular perfusion pressure (OPP), the difference between ocular arterial blood pressure and IOP, shows a significant correlation to the prevalence, incidence, and progression of the disease.[2]

Peak IOP is related to glaucoma progression, and previous studies have shown that IOP peaks of glaucoma patients frequently occur outside of office hours.[3] Different classes of glaucoma drugs have variable IOP lowering efficacies during the nocturnal/sleep period compared with their efficacies during the diurnal/wake period.[4-8] Prostaglandin analogues, such as latanoprost and travoprost, have been shown to be effective in lowering IOP during both the diurnal and nocturnal periods.[5,6] Bimatoprost (Lumigan, Allergan, Irvine, CA) is a prostaglandin $F_{2\alpha}$ analogue that also lowers IOP.[9] It appears to mimic the activity of prostaglandins and reduces IOP by enhancing uveoscleral and possibly trabecular outflow via direct effect on ciliary muscle relaxation and remodeling of extracellular matrix.[10] Bimatoprost 0.03% has been demonstrated to be safe and highly effective in lowering IOP over the long term in glaucoma and ocular hypertension.[11,12] The most common side effect of bimatoprost

and other prostaglandin analogues is conjunctival hyperemia, which can often lead to non-compliance or discontinuation of treatment.[13]

Bimatoprost 0.01% is a new formulation that was developed to improve the tolerability but maintaining the IOP-lowering effectiveness of bimatoprost 0.03% in lowering IOP. A recent study showed that bimatoprost 0.01% is equivalent to bimatoprost 0.03% in lowering IOP during the diurnal/wake period throughout 12 months of treatment with less associated incidence of side effects such as conjunctival hyperemia.[14] However, the nocturnal IOP lowering effect of bimatoprost 0.01% has not been investigated. In addition, the 24-hour effect of bimatoprost 0.01% on OPP is unknown. The present study evaluated the 24-hour effects on IOP and OPP of bimatoprost 0.01% monotherapy in a group of patients with POAG or ocular hypertension.

MATERIALS AND METHODS

The study was approved by the University of California, San Diego Institutional Review Board, in accordance with the Health Insurance Portability and Accountability Act, and registered as a clinical trial (http://www.clinicaltrials.gov). All methods adhered to the Declaration of Helsinki for research involving human subject. Experimental subjects were recruited consecutively from patients with diagnosed bilateral POAG or ocular hypertension at the Hamilton Glaucoma Center of the University of California, San Diego. All subjects enrolled in this study were between 40 to 80 years old with untreated IOP equal to or above 22 mmHg during office hours. Criteria for the diagnosis of glaucoma and ocular hypertension have been described previously.[5,15] In brief, patients with ocular hypertension had untreated IOP over 21 mm Hg during the most recent 2 or more office visits, but otherwise normal optic discs and visual fields. Patients with POAG had abnormalities in optic discs or visual fields (or both). Subjects were fully informed about this study, and informed consents were obtained.

Subjects who smoked, had previous glaucoma surgery in either eye, had a history of ocular trauma or a sleep disorder, or had an irregular sleep schedule were excluded. All subjects' medical history was reviewed and each subject had an eye examination, including slit-lamp biomicroscopy, gonioscopy, Goldmann applanation tonometry, (Haag-Streit, Mason, OH), dilated funduscopy, and a visual field test. Individuals with ocular inflammation, narrow iridocorneal angle, severe cardiovascular or diabetic condition, or use of a systemic β-blocker for treating high blood pressure were excluded. Routine systemic medications used by the subjects were documented and subjects with a change in the systemic medications during the study period would be excluded.

Subjects were either treatment naïve or washed out from a prior glaucoma medication 4 weeks prior to enrollment at the discretion of the ophthalmologist. Subjects were instructed to maintain a daily 8-hour regular sleep schedule for 1 week before the laboratory recording, and this 8-hour period was referred to as the nocturnal/sleep period. Individual sleep periods were verified using a wrist monitor for light exposure and arm movements (Actiwatch, Mini Mitter, Sunriver, OR) and a wake/sleep log. Subjects were asked to abstain from alcohol for 3 days and coffee for 1 day before the laboratory session beginning at approximately 2 PM. Baseline data of 24-hour IOP in a sleep laboratory were collected. Subjects were then treated with bimatoprost 0.01% in both eyes one time nightly approximately a half hour before bedtime. The bimatoprost 0.01% treatment lasted for 4 weeks before the second 24-hour laboratory recording.

Laboratory conditions and general experimental procedures have been described previously.[7] The 8-hour nocturnal/sleep period in the laboratory for each subject was adjusted to correspond to the recorded bedtime in the previous week. Clock times for the IOP measurements were also individualized. However, laboratory data were aligned as if each subject had a nocturnal/sleep period from 11 PM to 7 AM. The actual length of sleep in the laboratory may be less than 8 hours in some subjects. Subjects were encouraged to continue normal indoor activities in the laboratory. Food and water were available, and meal times were not regulated. During the second laboratory recording, subjects self-administered the bimatoprost 0.01% eyedrops under supervision at approximately 10:30 PM.

Intraocular pressure, blood pressure, and heart rate were measured every 2 hours. Experienced researchers performed the measurements in 3 random shifts. Their inter-

individual variations of IOP measurements were confirmed as insignificant. Intraocular pressure was measured using a calibrated pneumatonometer (Reichert, Depew, NY). Topical 0.5% proparacaine was used as the local anesthetic. Every plot of IOP measurement was evaluated according to commonly accepted standards.[16] Blood pressure and heart rate were measured immediately before the IOP measurements using an automated arm monitor (Accutorr Plus, Datascope Inc., Montvale, NJ). The cuff was mounted over the brachial artery level with the heart, and removed after the measurement. Before bedtime, measurements were taken at 3:30 PM, 5:30 PM, 7:30 PM, and 9:30 PM. Subjects were instructed to lie in bed for 5 minutes before the supine measurements and then to sit for 5 minutes before the sitting measurements. Lights in individual sleep rooms were turned off at 11 PM. Measurements were taken supine only at 11:30 PM, 1:30 AM, 3:30 AM, and 5:30 AM. Subjects were awakened, and the measurements were taken immediately. A dim red room light of less than 10 lux was used to assist the measurements. Some sleep disturbance was unavoidable because of the nocturnal measurements, and the levels of blood pressure and IOP may be influenced by the measurement procedure. However, both laboratory sessions are affected, and effects of sleep disturbance and nocturnal measurement procedures on IOP may be insignificant.[17,18] Room lighting was restored at 7 AM, and subjects were awakened. Measurements were taken at 7:30 AM, 9:30 AM, 11:30 AM, and 1:30 PM. Timings of the measurements were documented using infrared camera recording.

Data of IOP from both eyes were averaged. Mean arterial blood pressure was calculated as the diastolic blood pressure plus one third of the difference between the systolic and the diastolic blood pressures. Means of IOP, blood pressure, and heart rate

were calculated for the diurnal period (8 readings between 7 AM and 11 PM) and the nocturnal period (4 readings between 11 PM and 7 AM). Diurnal and nocturnal OPP in different body positions were calculated, using previously described formulae based on the mean blood pressure and IOP, adjusted for the height of the eye over the heart:[19]

Sitting OPP = 95/140 x mean blood pressure – IOP

Supine OPP = 115/130 x mean blood pressure – IOP

Statistical comparisons of study parameters were performed between the bimatoprost 0.01% treatment and the baseline using the paired t test. The criterion for statistical significance was $P<0.05$.

RESULTS

Sixteen subjects aged 49 to 77 years (61.0 ± 8.5 years, mean ± standard deviation), 10 male and 6 female, were recruited, and all completed the study. There were 11 Caucasians, 2 Asians, 2 blacks, and 1 Hispanic. Thirteen patients had an ocular hypertension diagnosis and 3 patients had a diagnosis POAG. The last office-hour IOP measured using the Goldmann tonometer under no treatment before the enrollment was 26.4 ± 3.7 mmHg.

Figure 1 shows the 24-hour profiles of habitual IOP (sitting during the day and supine at night) under the 2 experimental conditions. As shown, IOP increased significantly during the transition from the diurnal period to the nocturnal period when the body position changed from sitting to supine, and IOP decreased significantly during the transition from the nocturnal to the diurnal period. During the 16-hour diurnal period, the mean IOP under the bimatoprost 0.01% treatment was consistently lower than the baseline. During the 8-hour nocturnal period, mean IOP levels under the bimatoprost 0.01 % treatment were also lower than baseline. The IOP reduction was approximately 2 times during the diurnal period than during the nocturnal period. Figure 2 presents the 24-hour supine IOP profiles at baseline and under bimatoprost 0.01% treatment. Supine IOP under the bimatoprost 0.01% treatment was lower than the baseline during the diurnal period.

Table 1 summarizes the mean diurnal and nocturnal IOP levels, mean blood pressures, and mean OPP under the 2 experimental conditions. During the diurnal period, sitting and supine IOP under the bimatoprost 0.01% treatment were significantly lower than the baseline IOP in the same body position ($P<0.001$). During the nocturnal period,

the supine IOP under the bimatoprost 0.01% treatment was also significantly lower than the baseline IOP (P<0.01). There was no significant change in systolic, diastolic, or mean blood pressure with bimatoprost 0.01% treatment. During the diurnal period, OPP in the sitting or supine position was significantly greater with bimatoprost 0.01% treatment than at baseline (p<0.001). In the nocturnal period, the OPP with treatment was also significantly greater than baseline (p<0.05).

There was no significant change in the heart rate with bimatoprost 0.01% treatment in both body positions during the diurnal/wake period, but the supine heart rate during the nocturnal period was significantly reduced by 6.3 ± 9.2 beats/min under the treatment (P<0.05).

Table 1. Mean IOP, BP, and OPP Before and After Treatment With Bimatoprost 0.01%.

	Baseline	Post-Treatment	Difference	P-Value
Diurnal Sitting				
Mean IOP	22.6 ± 3.9	17.6 ± 2.5	-4.9 ± 3.5	<0.001
Mean BP	91.1 ± 9.4	91.6 ± 9.9	0.6 ± 5.3	0.67
Mean OPP	39.2 ± 7.4	44.5 ± 7.1	5.3 ± 3.8	<0.001
Diurnal Supine				
Mean IOP	27.0 ± 3.4	21.6 ± 2.0	-5.4 ± 3.1	<0.001
Mean BP	87.7 ± 8.9	87.4 ± 7.5	0.4 ± 5.2	0.75
Mean OPP	49.9 ± 9.0	55.7 ± 7.2	5.7 ± 4.6	<0.001
Nocturnal Supine				
Mean IOP	25.4 ± 4.8	22.8 ± 3.7	-2.6 ± 2.8	<0.01
Mean BP	83.6 ± 10.4	85.8 ± 7.9	2.2 ± 8.6	0.31
Mean OPP	48.5 ± 10.4	53.1 ± 6.6	4.6 ± 7.5	<0.05

All data in mmHg unit (mean ± standard deviation; N = 16)

Diurnal: 7 AM to 11 PM, Nocturnal: 11 PM to 7 AM

IOP = Intraocular pressure, BP = Blood pressure, OPP = Ocular perfusion pressure, P-value performed with paired t-test

DISCUSSION

Our results showed a significant IOP lowering effect of bimatoprost 0.01% monotherapy during both the diurnal and nocturnal periods. The nocturnal IOP lowering efficacy of bimatoprost 0.01% was less than that of the diurnal period, similar to previous studies with latanoprost and travoprost.[5,6] Posture was not a factor in the modulated nocturnal IOP lowering efficacy since IOP measurements under treatment in the supine position demonstrated greater IOP reduction during the wake period than at night.

Reduced OPP could enhance glaucomatous optic nerve damage. Primary open-angle glaucoma patients often have their lowest blood pressures at night, and a reduction in nocturnal blood pressure may lower perfusion pressure to ocular tissues.[20] The present study demonstrated that the systolic, diastolic, and mean blood pressures did not significantly change over the 24-hour period with bimatoprost 0.01% treatment compared to baseline. In contrast, the diurnal and nocturnal OPP significantly increased under bimatoprost 0.01% treatment, reflecting the fact that treatment significantly decreased IOP. This increase in OPP may have favorable implications in the treatment of glaucoma. However, the study population was skewed because we only included those patients with office-hour IOP measurements of 22 mmHg or greater. This selection bias allowed for a potentially greater reduction of IOP. Consequently, there may not be a significant effect of bimatoprost 0.01% on OPP during the diurnal or nocturnal period for patients with IOP less than 22 mmHg.

Bimatoprost 0.01% was formulated with the goal of improving the safety profile and tolerability of the medication while maintaining the IOP lowering effect of bimatoprost 0.03%. This would be achieved by lowering the concentration of bimatoprost

and increasing the concentration of benzalkonium chloride (0.05% to 0.2%), a commonly used preservative that increases corneal penetration and intraocular bioavailability of topically applied medications.[21] Whether or not the formulation used for bimatoprost 0.01% may have modified the time-dependent IOP lowering profile of bimatoprost 0.03% cannot be answered by the present study.[13,22-25]

In summary, bimatoprost 0.01% monotherapy results in IOP lowering both during the diurnal/wake and nocturnal/sleep periods, with the IOP lowering effect being greater in the waking hours. Moreover, bimatoprost 0.01% significantly increases OPP over the 24-hour period.

Competing interests: John H.K. Liu, PhD has received support for research from Alcon and Allergan. Robert N. Weinreb, MD is a consultant for Alcon, Allergan, Bausch & Lomb, Merck Research Laboratories, and Pfizer and has received lecture fees from Alcon and Allergan. Felipe A. Medeiros, MD, PhD is a consultant for Alcon, Allergan, and Pfizer and has received lecture fees from Allergan and Pfizer.

Funding: This study was supported by a research grant (JHKL) from Allergan and an unrestricted grant from Research to Prevent Blindness (New York, NY).

Ethics approval: This study was approved by the University of California, San Diego Institutional Review Board.

Contributors: All authors made a significant contribution in designing the study, performing the experiments, evaluating the results, and writing the manuscript.

REFERENCES

1. **Weinreb RN**, Khaw PT. Primary open-angle glaucoma. *Lancet* 2004;**363**:1711-20.
2. **Weinreb RN**, Harris A. Ocular Blood Flow in Glaucoma. WGA Consensus Series 6. Amsterdam, the Netherlands: Kugler Publications, 2009:59-128.
3. **Bagga H**, Liu JH, Weinreb RN. Intraocular pressure measurements throughout the 24 h. *Curr Opin Ophthalmol* 2009;**20**:79-83.
4. **Orzalesi N**, Rossetti L, Invernizzi T, et al. Effect of timolol, latanoprost, and dorzolamide on circadian IOP in glaucoma or ocular hypertension. *Invest Ophthalmol Vis Sci* 2000;**41**:2566-73.
5. **Liu JH**, Kripke DF, Weinreb RN. Comparison of the nocturnal effects of once-daily timolol and latanoprost on intraocular pressure. *Am J Ophthalmol* 2004;**138**:389-95.
6. **Sit AJ**, Weinreb RN, Crowston JG, et al. Sustained effect of travoprost on diurnal and nocturnal intraocular pressure. *Am J Ophthalmol* 2006;**141**:1131-3.
7. **Liu JH**, Medeiros FA, Slight JR, et al. Comparing diurnal and nocturnal effects of brinzolamide and timolol on intraocular pressure in patients receiving latanoprost monotherapy. *Ophthalmology* 2009;**116**:449-54.
8. **Liu JH**, Medeiros FA, Slight JR, et al. Diurnal and nocturnal effects of brimonidine monotherapy on intraocular pressure. *Ophthalmology* 2010;**117**:2075-9.
9. **Woodward DF**, Phelps RL, Krauss AH, et al. Bimatoprost: a novel antiglaucoma agent. *Cardiovasc Drug Rev* 2004;**22**:103-20.

10. **Toris CB**, Gabelt BT, Kaufman PL. Update on the mechanism of action of topical prostaglandins for intraocular pressure reduction. *Surv Ophthalmol* 2008;**53** Suppl1:S107-20.
11. **Williams RD**, Cohen JS, Gross RL, et al. Long-term efficacy and safety of bimatoprost for intraocular pressure lowering in glaucoma and ocular hypertension: year 4. *Br J Ophthalmol* 2008;**92**:1387-92.
12. **Aptel F**, Cucherat M, Denis P. Efficacy and tolerability of prostaglandin analogs: a meta-analysis of randomized controlled clinical trials. *J Glaucoma* 2008;**17**:667-73.
13. **Konstas AG**, Katsimbris JM, Lallos N, et al. Latanoprost 0.005% versus bimatoprost 0.03% in primary open-angle glaucoma patients. *Ophthalmology* 2005;**112**:262-6.
14. **Katz LJ**, Cohen JS, Batoosingh AL, et al. Twelve-month, randomized, controlled trial of bimatoprost 0.01%, 0.0125%, and 0.03% in patients with glaucoma or ocular hypertension. *Am J Ophthalmol* 2010;**149**:661-71 e1.
15. **Liu JH**, Zhang X, Kripke DF, et al. Twenty-four-hour intraocular pressure pattern associated with early glaucomatous changes. *Invest Ophthalmol Vis Sci* 2003;**44**:1586-90.
16. **Liu JH**, Kripke DF, Twa MD, et al. Twenty-four-hour pattern of intraocular pressure in the aging population. *Invest Ophthalmol Vis Sci* 1999;**40**:2912-7.
17. **Mansouri K**, Shaarawy T. Continuous intraocular pressure monitoring with a wireless ocular telemetry sensor: initial clinical experience in patients with open angle glaucoma. *Br J Ophthalmol* 2011;**95**:627-9.

18. **Liu JH**, Weinreb RN. Monitoring intraocular pressure for 24 h. *Br J Ophthalmol* 2011;**95**:599-600.

19. **Liu JH**, Gokhale PA, Loving RT, et al. Laboratory assessment of diurnal and nocturnal ocular perfusion pressures in humans. *J Ocul Pharmacol Ther* 2003;**19**:291-7.

20. **Quaranta L**, Gandolfo F, Turano R, et al. Effects of topical hypotensive drugs on circadian IOP, blood pressure, and calculated diastolic ocular perfusion pressure in patients with glaucoma. *Invest Ophthalmol Vis Sci* 2006;**47**:2917-23.

21. **Nakamura T**, Yamada M, Teshima M, et al. Electrophysiological characterization of tight junctional pathway of rabbit cornea treated with ophthalmic ingredients. *Biol Pharm Bull* 2007;**30**:2360-4.

22. **Orzalesi N**, Rossetti L, Bottoli A, et al. Comparison of the effects of latanoprost, travoprost, and bimatoprost on circadian intraocular pressure in patients with glaucoma or ocular hypertension. *Ophthalmology* 2006;**113**:239-246.

23. **Yildirim N**, Sahin A, Gultekin S. The effect of latanoprost, bimatoprost, and travoprost on circadian variation of intraocular pressure in patients with open-angle glaucoma. *J Glaucoma* 2008;**17**:36-9.

24. **Quaranta L**, Pizzolante T, Riva I, et al. Twenty-four-hour intraocular pressure and blood pressure levels with bimatoprost versus latanoprost in patients with normal-tension glaucoma. *Br J Ophthalmol* 2008;**92**:1227-31.

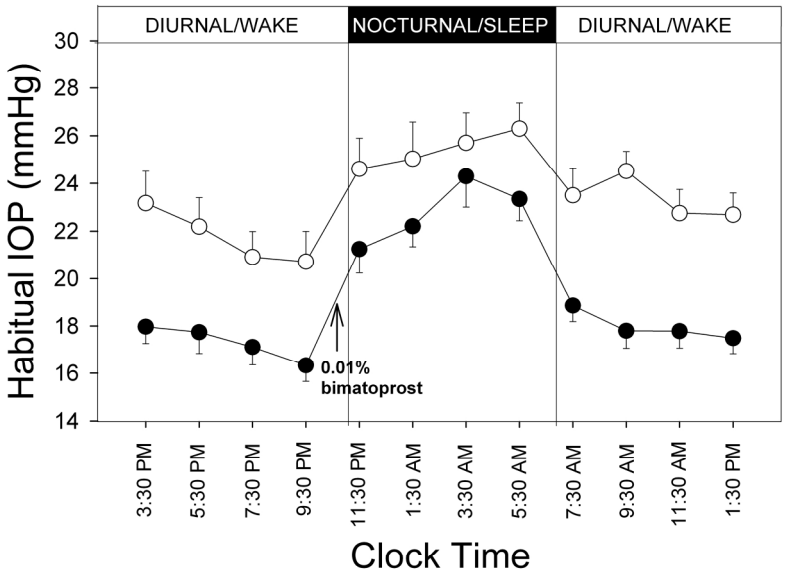
25. **Konstas AG**, Hollo G, Mikropoulos D, et al. Twenty-four-hour intraocular pressure control with bimatoprost and the bimatoprost/timolol fixed combination

administered in the morning, or evening in exfoliative glaucoma. *Br J Ophthalmol* 2010;**94**:209-13.

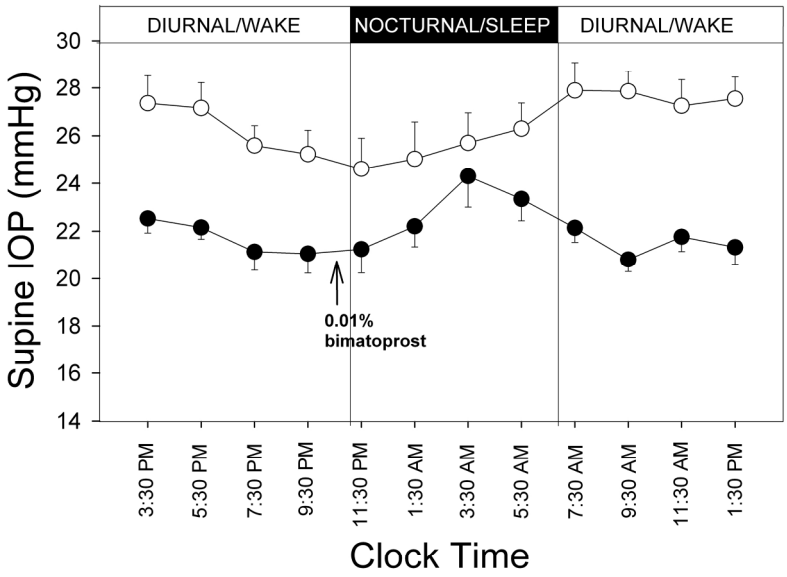
FIGURE LEGENDS

Figure 1. Profiles of 24-hour IOP in the habitual body positions. Measurements were taken from 16 subjects sitting during the diurnal period and supine during the nocturnal period. Open circles represent the baseline, and solid circles represent the bimatoprost 0.01% treatment for 4 weeks. Error bars represent standard error of the mean. IOP = intraocular pressure.

Figure 2. Profiles of 24-hour supine IOP. Open circles represent the baseline, and solid circles represent the bimatoprost 0.01% treatment for 4 weeks. Data were from the same 16 subjects as in Figure 1. Error bars represent standard error of the mean. IOP = intraocular pressure.



215x166mm (300 x 300 DPI)



215x166mm (300 x 300 DPI)

BMJ open

Twenty-four-hour effects of bimatoprost 0.01% monotherapy on intraocular pressure and ocular perfusion pressure

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2012-001106.R1
Article Type:	Research
Date Submitted by the Author:	16-Jun-2012
Complete List of Authors:	Tung, Jonathan; University of California, San Diego, Ophthalmology Tafreshi, Ali; University of California, San Diego, Ophthalmology Weinreb, Robert; University of California, San Diego, Ophthalmology Slight, J; University of California, San Diego, Ophthalmology Medeiros, Felipe; University of California, San Diego, Ophthalmology Liu, John; University of California, San Diego, Ophthalmology
Primary Subject Heading:	Ophthalmology
Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	Glaucoma < OPHTHALMOLOGY, CLINICAL PHARMACOLOGY, Clinical trials < THERAPEUTICS

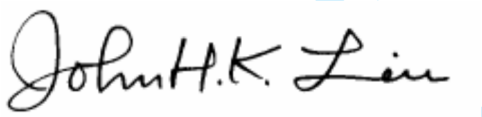
June 15, 2012

Mr. Richard Sands
Managing Editor, BMJ Open

Dear Mr. Sands:

We thank you, the Editor-in-Chief, and the reviewers for very valuable comments. All the issues raised have been addressed in the revised manuscript. Our point-by-point responses are enclosed. We hope that the revised manuscript will have the approval for publication in BMJ Open.

Sincerely,



John H.K. Liu, PhD
Corresponding Author

From the editor-in-chief
It's a tiny case series with selected patients but, as long as the authors discuss it very cautiously, it's publishable and within scope for BMJ Open.

Please ensure the manuscript addresses the following issues:
* did the study use predefined, clearly described inclusion and exclusion criteria?

We clarified in the first paragraph of Materials and Methods that this is a prospective study with predefined inclusion and exclusion criteria.

* Please report the setting/context/wider population in detail eg how representative was the hospital of other such units? how are patients referred there (if it's a tertiary service they will have been referred by GPs to specialists and then on to super-specialists and will be highly unrepresentative)?

The recruitment site is a tertiary ophthalmic clinic, which was clarified in the first paragraph of Materials and Methods. It is common in the USA that diagnosis and treatment of glaucoma occurs in a tertiary ophthalmic clinic.

* was there a fixed period for sampling? If so, how many eligible patients were not included in the study during that period? How did they differ from the participants?

The recruitment occurred between January and August, 2011. This information was added to the first paragraph in Results. It is our impression that approximately half of the eligible candidates were approached but were not enrolled. The major reason was the difficulty for a patient to commit to two 24-hour laboratory recordings. The same inclusion and exclusion criteria were applied to all eligible study candidates.

*was the study prospective or retrospective (ie case details came from routine records that were probably collated inconsistently, introducing bias)?

This is a prospective study.

From the managing editor:
Please use a structured abstract as explained here:
http://bmjopen.bmj.com/site/about/guidelines.xhtml#research

Abstract was revised accordingly.

The funding statement should fully describe the role of the study sponsors; provide a statement on the independence of researchers from funders; and state whether all authors had full access to and can take responsibility for the data and analyses.

The following two statements were added at the end of the text;
"Allergan has no control or influence on this study" under Funding and "All authors had full access to and can take responsibility for the data and analyses" under Contributors.

Reviewer: E. Randy Craven, MD
Associate Clinical Professor, Rocky Vista University
Speaker and Consultant for Allergan
Speaker for Merck
Speaker for Alcon

Not clear why this would be Cardiovascular, dermatology and anesthesia as searchable words. They don't fit at all

Appropriate headings and keywords were added.

Reviewer: Luca Rossetti, MD
Director Eye Clinic, University of Milan at San Paolo Hospital, Milan, Italy

Consultant for Alcon, Allergan, MSD, Baush and Lomb, Pfizer

The paper "Twenty-four-hour effects of bimatoprost 0.01%monotherapy on intraocular pressure and ocular perfusion pressure" is about the effect of bimatoprost on 24-hour IOP and OPP. The study methodology is sound and findings seem to be interesting. I have a few comments about this paper and are following

- Patients: how was the sample of 16 subjects considered? Was it based on any calculation on bimatoprost "potential effect" on 24-hour IOP?

A pre-study calculation of sample size was performed when we applied the research funding. An anticipated standard deviation of the nocturnal IOP difference (2 mmHg) between the two experimental conditions and a desirable nocturnal IOP-lowering effect (1.6 mmHg) were used based upon our previous experience with IOP-lowering drugs including other prostaglandin analogs. When accepting the statistical power of 0.80 and 0.05 Type I error, the calculated sample size was 15. We enrolled 16 subjects with caution that one subject may not complete both 24-hour laboratory recordings. Please note that this pre-study sample calculation was not presented since the real data variation (not the assumption as shown before) was presented in the manuscript and the results were positive.

- Patients: a Table reporting patients' main characteristics should be presented. In particular, as many of the subjects had ocular hypertension, how was CCT?

A new Table with patients' demographic information was added. Also, we deleted the redundant information from the text.

- Patients: some of the subjects were newly diagnosed, while others were treated and underwent a drug wash-out before inclusion. Numbers should be presented.

14 patients were newly diagnosed and 2 existing patients underwent washout. This information was added to the first paragraph of Results.

- Patients: for those who were on therapy, type of drugs should be reported. Were there any patients treated with 0.03% bimatoprost?

Information was added to the first paragraph in Results. No patient was treated with bimatoprost before the study.

- Patients: the vast majority of the cases had ocular hypertension and only 3 had glaucoma. This could have a potential effect on the results generalizability to the glaucoma population. This needs to be discussed.

Discussion of this issue was added to the 2nd paragraph of Discussion (last four sentences).

- Stats: baseline and data after treatment were compared with paired-t test, which is a parametric test. Was normality of data distribution checked? Wilcoxon (non-parametric) test is usually applied when sample size is limited.

Thank you for this reminder. Normal distribution of test data was confirmed using the Kolmogorov-Smirnov test. This information was added at the end of Materials and Methods.

- Results: Figure 1 is missing from the paper pdf file.

This problem will be checked for the revision.

Reviewer: Andreas Katsanos
Ophthalmology Department
University of Ioannina, Ioannina, Greece

The authors have examined the ocular hypotensive effect and the effect on the calculated ocular perfusion pressure of the recently introduced bimatoprost 0.01% solution.
Following are a number of issues that need to be addressed.

Introduction, 2nd paragraph, 1st line. The authors should support their statement that "peak IOP is related to glaucoma progression" with relevant literature.

A new reference (a review/consensus article) was added as Reference 3.

Materials & methods, 1st paragraph. I would recommend mentioning the clinicaltrials.gov identifier.

Number of identifier (NCT01271686) was added.

Materials & methods, page 8, 1st line. Did the authors use any formal way of determining measurement variability? How exactly were "…the variations of IOP measurements confirmed as insignificant"?

We used ±2 mmHg as the guideline to test the variability. It was added to the text. This number is used in the manufacturer manual to verify the accuracy of the instrument.

Results, page 11, last lines. Can the authors offer an explanation regarding the nocturnal reduction of heart rate at the supine position after bimatoprost treatment?

The real reason is unknown. We suspect that the study order of baseline-treatment may be a factor. However, we prefer not adding the speculation to the text.

The authors have published several interesting papers in the past (eg Liu, Kripke et al IOVS 1998; Liu, Zhang et al IOVS 2003; Liu, Bouligny et al IOVS 2003) showing that nocturnal IOP readings are higher than diurnal readings, at least in healthy participants. In the current study, baseline supine readings at night are lower than daytime readings. This finding fits well with the more traditional understanding that in general, IOP tends to be higher in the morning. In addition, figure 2 of the currently submitted manuscript seems quite similar with figure 1 of the Liu, Zhang et al paper (IOVS 2003;44:1586), in which glaucoma patients have a morning, rather than a night-time peak. The authors are encouraged to comment on the potentially different IOP peaks that healthy individuals and POAG patients may have.

In the present study, there were 13 patients with ocular hypertension and only 3 with primary open-angle glaucoma (POAG). The 2003 article by Liu et al specifically showed the 24-hour IOP pattern in patients with early glaucomatous changes. The potentially different IOP peaks in the healthy individuals and POAG has been discussed in that article. To the best of our knowledge, there is no published paper that shows the 24-hour IOP pattern in patients with ocular hypertension. We agree with the reviewer that this is an important area for research and look forward to seeing relevant publications.

It could be worth mentioning in the discussion that nocturnal & diurnal efficacy is related, at least in part, to the time of drug instillation and the peak/trough efficacy of the medication.

We added the following sentence "It is possible that the difference in diurnal and nocturnal efficacies is related to the pharmacokinetics of prostaglandin analogs given once a day in the evening" to the first paragraph of Discussion.

Twenty-Four-Hour Effects of Bimatoprost 0.01% Monotherapy On Intraocular Pressure and Ocular Perfusion Pressure

Jonathan D. Tung, MD

Ali Tafreshi, BSc

Robert N. Weinreb, MD

J. Rigby Slight, MD

Felipe A. Medeiros, MD, PhD

John H.K. Liu, PhD

Hamilton Glaucoma Center and Department of Ophthalmology, University of California, San Diego, La Jolla, California, USA.

Space count for title: 111. Word count for abstract: 204~~194~~. Word count for text: 2171~~067~~.

Running head: 24-hour effects of bimatoprost 0.01%.

Tables and figures: 2~~1~~ tables and 2 figures.

Corresponding author: John H.K. Liu, PhD, University of California, San Diego, Department of Ophthalmology, La Jolla, CA 92093-0946, USA.

Phone: (858) 534-7056. Fax: (858) 534-7014. E-mail: joliu@ucsd.edu.

ABSTRACT

ObjectivesAim: To investigate the 24-hour effects of bimatoprost 0.01% monotherapy on intraocular pressure (IOP) and ocular perfusion pressure (OPP).

Design: in a pProspective, open-label experimental study.

Setting: Single tertiary ophthalmic clinic.

ParticipantsMethods: Sixteen patients with diagnosed primary open-angle glaucoma (POAG) or ocular hypertension (ages, 49–77 years) were enrolled.

Interventions: Baseline data of 24-hour IOP in untreated patients were collected in a sleep laboratory. Measurements of IOP were taken using a pneumatonometer every 2 hours in the sitting and supine body positions during the 16-hour diurnal/wake period and in the supine position during the 8-hour nocturnal/sleep period. After baseline measurements were taken, patients were treated with bimatoprost 0.01% one time per day at bedtime for 4 weeks, and then 24-hour IOP data were collected under the same laboratory conditions.

Primary and secondary outcome measures: Diurnal and nocturnal IOP and OPP means under bimatoprost 0.01% treatment were compared with baseline.

Results: The diurnal and nocturnal IOP means were significantly lower under the bimatoprost 0.01% treatment than baseline in both the sitting and supine positions. The diurnal and nocturnal OPP means were significantly higher under treatment than baseline in both the sitting and supine positions.

Conclusion: Bimatoprost 0.01% monotherapy significantly lowered IOP and increased OPP during the 24-hour period.

ARTICLE SUMMARY

Article focus:

- Diurnal and nocturnal effects of a new bimatoprost formulation.

Key messages:

- Bimatoprost 0.01% lowers intraocular pressure.
- Bimatoprost 0.01% increases ocular perfusion pressure.

Strengths and limitations of this study:

- Experimental study under strictly controlled conditions.
- Small sample size.

INTRODUCTION

Glaucoma is a progressive optic neuropathy characterized by pathologic loss of retinal ganglion cells and retinal nerve fiber layer with associated visual field loss. The lowering of intraocular pressure (IOP) is the only proven method to reduce the risk of glaucoma onset and slow disease progression.[1] Studies have also shown an association between primary open-angle glaucoma (POAG) and vascular factors, such as systemic hypertension, hypotension, vasospasm, atherosclerosis, and ocular blood flow.[1,2] While the association between systemic blood pressure and POAG is weak, ocular perfusion pressure (OPP), the difference between ocular arterial blood pressure and IOP, shows a significant correlation to the prevalence, incidence, and progression of the disease.[2]

Peak IOP is related to glaucoma progression, and previous studies have shown that IOP peaks of glaucoma patients frequently occur outside of office hours.[3,4] Different classes of glaucoma drugs have variable IOP lowering efficacies during the nocturnal/sleep period compared with their efficacies during the diurnal/wake period.[5~~4~~-9~~8~~] Prostaglandin analogues, such as latanoprost and travoprost, have been shown to be effective in lowering IOP during both the diurnal and nocturnal periods.[~~5,~~6,7] Bimatoprost (Lumigan, Allergan, Irvine, CA) is a prostaglandin $F_{2\alpha}$ analogue that also lowers IOP.[10~~9~~] It appears to mimic the activity of prostaglandins and reduces IOP by enhancing uveoscleral and possibly trabecular outflow via direct effect on ciliary muscle relaxation and remodeling of extracellular matrix.[11~~10~~] Bimatoprost 0.03% has been demonstrated to be safe and highly effective in lowering IOP over the long term in glaucoma and ocular hypertension.[~~11,~~12,13] The most common side effect of

bimatoprost and other prostaglandin analogues is conjunctival hyperemia, which can often lead to non-compliance or discontinuation of treatment.[143]

Bimatoprost 0.01% is a new formulation that was developed to improve the tolerability but maintaining the IOP-lowering effectiveness of bimatoprost 0.03% in lowering IOP. A recent study showed that bimatoprost 0.01% is equivalent to bimatoprost 0.03% in lowering IOP during the diurnal/wake period throughout 12 months of treatment with less associated incidence of side effects such as conjunctival hyperemia.[154] However, the nocturnal IOP lowering effect of bimatoprost 0.01% has not been investigated. In addition, the 24-hour effect of bimatoprost 0.01% on OPP is unknown. The present study evaluated the 24-hour effects on IOP and OPP of bimatoprost 0.01% monotherapy in a group of patients with POAG or ocular hypertension.

MATERIALS AND METHODS

Thise prospective study was approved by the University of California, San Diego Institutional Review Board, in accordance with the Health Insurance Portability and Accountability Act, and registered as a clinical trial (http://www.clinicaltrials.gov; NCT01271686). All methods adhered to the Declaration of Helsinki for research involving human subject. Experimental subjects were recruited consecutively from patients with diagnosed bilateral POAG or ocular hypertension at the Hamilton Glaucoma Center of the University of California, San Diego, a tertiary ophthalmic clinic, with predefined inclusion and exclusion criteria. All subjects enrolled in this study were between 40 to 80 years old with untreated IOP equal to or above 22 mmHg during office hours. Criteria for the diagnosis of glaucoma and ocular hypertension have been described previously.[65,165] In brief, patients with ocular hypertension had untreated IOP over 21 mm Hg during the most recent 2 or more office visits, but otherwise normal optic discs and visual fields. Patients with POAG had abnormalities in optic discs or visual fields (or both). Subjects were fully informed about this study, and informed consents were obtained.

Subjects who smoked, had previous glaucoma surgery in either eye, had a history of ocular trauma or a sleep disorder, or had an irregular sleep schedule were excluded. All subjects' medical history was reviewed and each subject had an eye examination, including slit-lamp biomicroscopy, gonioscopy, Goldmann applanation tonometry, (Haag-Streit, Mason, OH), dilated funduscopy, and a visual field test. Individuals with ocular inflammation, narrow iridocorneal angle, severe cardiovascular or diabetic condition, or use of a systemic β-blocker for treating high blood pressure were excluded.

Routine systemic medications used by the subjects were documented and subjects with a change in the systemic medications during the study period would be excluded.

Subjects were either treatment naïve or washed out from a prior glaucoma medication 4 weeks prior to enrollment at the discretion of the ophthalmologist. Subjects were instructed to maintain a daily 8-hour regular sleep schedule for 1 week before the laboratory recording, and this 8-hour period was referred to as the nocturnal/sleep period. Individual sleep periods were verified using a wrist monitor for light exposure and arm movements (Actiwatch, Mini Mitter, Sunriver, OR) and a wake/sleep log. Subjects were asked to abstain from alcohol for 3 days and coffee for 1 day before the laboratory session beginning at approximately 2 PM. Baseline data of 24-hour IOP in a sleep laboratory were collected. Subjects were then treated with bimatoprost 0.01% in both eyes one time nightly approximately a half hour before bedtime. The bimatoprost 0.01% treatment lasted for 4 weeks before the second 24-hour laboratory recording.

Laboratory conditions and general experimental procedures have been described previously.[8~~7~~] The 8-hour nocturnal/sleep period in the laboratory for each subject was adjusted to correspond to the recorded bedtime in the previous week. Clock times for the IOP measurements were also individualized. However, laboratory data were aligned as if each subject had a nocturnal/sleep period from 11 PM to 7 AM. The actual length of sleep in the laboratory may be less than 8 hours in some subjects. Subjects were encouraged to continue normal indoor activities in the laboratory. Food and water were available, and meal times were not regulated. During the second laboratory recording, subjects self-administered the bimatoprost 0.01% eyedrops under supervision at approximately 10:30 PM.

Intraocular pressure, blood pressure, and heart rate were measured every 2 hours. Experienced researchers performed the measurements in 3 random shifts. Their inter-individual variations of IOP measurements were confirmed as insignificant (within ± 2 mmHg). Intraocular pressure was measured using a calibrated pneumatonometer (Reichert, Depew, NY). Topical 0.5% proparacaine was used as the local anesthetic. Every plot of IOP measurement was evaluated according to commonly accepted standards.[176] Blood pressure and heart rate were measured immediately before the IOP measurements using an automated arm monitor (Accutorr Plus, Datascope Inc., Montvale, NJ). The cuff was mounted over the brachial artery, level with the heart, and removed after the measurement. Before bedtime, measurements were taken at 3:30 PM, 5:30 PM, 7:30 PM, and 9:30 PM. Subjects were instructed to lie in bed for 5 minutes before the supine measurements and then to sit for 5 minutes before the sitting measurements. Lights in individual sleep rooms were turned off at 11 PM. Measurements were taken supine only at 11:30 PM, 1:30 AM, 3:30 AM, and 5:30 AM. Subjects were awakened, and the measurements were taken immediately. A dim red room light of less than 10 lux was used to assist the measurements. Some sleep disturbance was unavoidable because of the nocturnal measurements, and the levels of blood pressure and IOP may be influenced by the measurement procedure. However, both laboratory sessions are affected, and effects of sleep disturbance and nocturnal measurement procedures on IOP may be insignificant.[17,18,19] Room lighting was restored at 7 AM, and subjects were awakened. Measurements were taken at 7:30 AM, 9:30 AM, 11:30 AM, and 1:30 PM. Timings of the measurements were documented using infrared camera recording.

Data of IOP from both eyes were averaged. Mean arterial blood pressure was calculated as the diastolic blood pressure plus one third of the difference between the systolic and the diastolic blood pressures. Means of IOP, blood pressure, and heart rate were calculated for the diurnal period (8 readings between 7 AM and 11 PM) and the nocturnal period (4 readings between 11 PM and 7 AM). Diurnal and nocturnal OPP in different body positions were calculated, using previously described formulae based on the mean blood pressure and IOP, adjusted for the height of the eye over the heart:[20~~19~~]

Sitting OPP = 95/140 x mean blood pressure – IOP

Supine OPP = 115/130 x mean blood pressure – IOP

Statistical comparisons of study parameters were performed between the bimatoprost 0.01% treatment and the baseline using the paired t test. Normal distribution of test data was confirmed using the Kolmogorov-Smirnov test. The criterion for statistical significance was $P<0.05$.

RESULTS

Sixteen subjects ~~aged 49 to 77 years (61.0 ± 8.5 years, mean ± standard deviation), 10 male and 6 female,~~ were recruited between January and August, 2011, and all completed the study. Their demographic data are presented in Table 1. ~~There were 11 Caucasians, 2 Asians, 2 blacks, and 1 Hispanic.~~ Thirteen patients had an ocular hypertension diagnosis and three~~3~~ patients had a POAG diagnosis ~~POAG~~. Fourteen patients were newly diagnosed and untreated before the enrollment. One patient with ocular hypertension was treated with latanoprost and the other patient with ocular hypertension was treated with dorzolamide. These two patients underwent 4-week washout before the first laboratory recording. The last office-hour IOP measured using the Goldmann tonometer under no treatment before the laboratory recording~~enrollment~~ was 26.4 ± 3.7 mmHg (mean ± standard deviation; range 22-36 mmHg).

Figure 1 shows the 24-hour profiles of habitual IOP (sitting during the day and supine at night) under the 2 experimental conditions. As shown, IOP increased significantly during the transition from the diurnal period to the nocturnal period when the body position changed from sitting to supine, and IOP decreased significantly during the transition from the nocturnal to the diurnal period. During the 16-hour diurnal period, the mean IOP under the bimatoprost 0.01% treatment was consistently lower than the baseline. During the 8-hour nocturnal period, mean IOP levels under the bimatoprost 0.01 % treatment were also lower than baseline. The IOP reduction was approximately 2 times during the diurnal period than during the nocturnal period. Figure 2 presents the 24-hour supine IOP profiles at baseline and under bimatoprost 0.01% treatment. Supine IOP

under the bimatoprost 0.01% treatment was lower than the baseline during the diurnal period.

Table 2~~1~~ summarizes the mean diurnal and nocturnal IOP levels, mean blood pressures, and mean OPP under the 2 experimental conditions. During the diurnal period, sitting and supine IOP under the bimatoprost 0.01% treatment were significantly lower than the baseline IOP in the same body position ($P<0.001$). During the nocturnal period, the supine IOP under the bimatoprost 0.01% treatment was also significantly lower than the baseline IOP ($P<0.01$). There was no significant change in systolic, diastolic, or mean blood pressure with bimatoprost 0.01% treatment. During the diurnal period, OPP in the sitting or supine position was significantly greater with bimatoprost 0.01% treatment than at baseline ($p<0.001$). In the nocturnal period, the OPP with treatment was also significantly greater than baseline ($p<0.05$).

There was no significant change in the heart rate with bimatoprost 0.01% treatment in both body positions during the diurnal/wake period, but the supine heart rate during the nocturnal period was significantly reduced by 6.3 ± 9.2 beats/min under the treatment ($P<0.05$).

Table 1. Demographic characteristics of 16 study participants.

Gender	10 male, 6 female
Ancestry	11 White, 2 Asian, 2 Black, 1 Hispanic
Age (year)	61.0 ± 8.5 (49-77)
Height (in.)	67.3 ± 3.9 (61-74)
Weight (lb.)	177.1 ± 35.4 (125-232)
Body Mass Index (lb./in.^{2})	27.4 ± 4.5 (19.6-33.8)
Central corneal thickness (μm)	591.0 ± 56.0 (490-695)

Values are the mean ± standard deviation (range).

Table 2~~1~~. Mean IOP, BP, and OPP before and after treatment with bimatoprost 0.01%.

	Baseline	Post-Treatment	Difference	P-Value
Diurnal Sitting				
Mean IOP	22.6 ± 3.9	17.6 ± 2.5	-4.9 ± 3.5	<0.001
Mean BP	91.1 ± 9.4	91.6 ± 9.9	0.6 ± 5.3	0.67
Mean OPP	39.2 ± 7.4	44.5 ± 7.1	5.3 ± 3.8	<0.001
Diurnal Supine				
Mean IOP	27.0 ± 3.4	21.6 ± 2.0	-5.4 ± 3.1	<0.001
Mean BP	87.7 ± 8.9	87.4 ± 7.5	0.4 ± 5.2	0.75
Mean OPP	49.9 ± 9.0	55.7 ± 7.2	5.7 ± 4.6	<0.001
Nocturnal Supine				
Mean IOP	25.4 ± 4.8	22.8 ± 3.7	-2.6 ± 2.8	<0.01
Mean BP	83.6 ± 10.4	85.8 ± 7.9	2.2 ± 8.6	0.31
Mean OPP	48.5 ± 10.4	53.1 ± 6.6	4.6 ± 7.5	<0.05

All data in mmHg unit (mean ± standard deviation; N = 16)

Diurnal: 7 AM to 11 PM, Nocturnal: 11 PM to 7 AM

IOP = Intraocular pressure, BP = Blood pressure, OPP = Ocular perfusion pressure, P-value performed with paired t-test

DISCUSSION

Our results showed a significant IOP lowering effect of bimatoprost 0.01% monotherapy during both the diurnal and nocturnal periods. The nocturnal IOP lowering efficacy of bimatoprost 0.01% was less than that of the diurnal period, similar to previous studies with latanoprost and travoprost.[~~5~~,6,7] Posture was not a factor in the modulated nocturnal IOP lowering efficacy since IOP measurements under treatment in the supine position demonstrated greater IOP reduction during the wake period than at night. It is possible that the difference in diurnal and nocturnal efficacies is related to the pharmacokinetics of prostaglandin analogs given once a day in the evening.

Reduced OPP could enhance glaucomatous optic nerve damage. Primary open-angle glaucoma patients often have their lowest blood pressures at night, and a reduction in nocturnal blood pressure may lower perfusion pressure to ocular tissues.[2~~0~~] The present study demonstrated that the systolic, diastolic, and mean blood pressures did not significantly change over the 24-hour period with bimatoprost 0.01% treatment compared to baseline. In contrast, the diurnal and nocturnal OPP significantly increased under bimatoprost 0.01% treatment, reflecting the fact that treatment significantly decreased IOP. This increase in OPP may have favorable implications in the treatment of glaucoma. However, the study population was skewed because we only included those patients with office-hour IOP measurements of 22 mmHg or greater. Most patients (13 of 16) enrolled in the present study were with ocular hypertension, not with POAG. This selection bias allowed for a potentially greater reduction of IOP. Consequently, there may not be a significant effect of bimatoprost 0.01% on OPP during the diurnal or nocturnal period for glaucoma patients with IOP less than 22 mmHg.

Bimatoprost 0.01% was formulated with the goal of improving the safety profile and tolerability of the medication while maintaining the IOP lowering effect of bimatoprost 0.03%. This would be achieved by lowering the concentration of bimatoprost and increasing the concentration of benzalkonium chloride (0.05% to 0.2%), a commonly used preservative that increases corneal penetration and intraocular bioavailability of topically applied medications.[22~~1~~] Whether or not the formulation used for bimatoprost 0.01% may have modified the time-dependent IOP lowering profile of bimatoprost 0.03% cannot be answered by the present study.[14~~3~~,23~~2~~-26~~5~~]

In summary, bimatoprost 0.01% monotherapy results in IOP lowering both during the diurnal/wake and nocturnal/sleep periods, with the IOP lowering effect being greater in the waking hours. Moreover, bimatoprost 0.01% significantly increases OPP over the 24-hour period.

Competing interests: John H.K. Liu, PhD has received support for research from Alcon and Allergan. Robert N. Weinreb, MD is a consultant for Alcon, Allergan, Bausch & Lomb, Merck Research Laboratories, and Pfizer and has received lecture fees from Alcon and Allergan. Felipe A. Medeiros, MD, PhD is a consultant for Alcon, Allergan, and Pfizer and has received lecture fees from Allergan and Pfizer.

Funding: This study was supported by an independent research grant (JHKL) from Allergan and an unrestricted grant from Research to Prevent Blindness (New York, NY). Allergan has no control or influence on this study.

Ethics approval: This study was approved by the University of California, San Diego Institutional Review Board.

Contributors: All authors made a significant contribution in designing the study, performing the experiments, evaluating the results, and writing the manuscript. All authors had full access to and can take responsibility for the data and analyses.

REFERENCES

1. **Weinreb RN**, Khaw PT. Primary open-angle glaucoma. *Lancet* 2004;**363**:1711-20.
2. **Weinreb RN**, Harris A. Ocular Blood Flow in Glaucoma. WGA Consensus Series 6. Amsterdam, the Netherlands: Kugler Publications, 2009:59-128.
3. **Medeiros FA**, Brandt J, Liu J, et al. IOP as a risk factor for glaucoma development and progression. In: Weinreb RN, Brandt JD, Garway-Heath DF, Medeiros FA, eds. Intraocular pressure: reports and consensus statements of the 4th Global AIGS Consensus Meeting on intraocular pressure. The Hague, The Netherlands: Kugler; 2007:59-74. WGA Consensus Series 4.

~~3.~~4. **Bagga H**, Liu JH, Weinreb RN. Intraocular pressure measurements throughout the 24 h. *Curr Opin Ophthalmol* 2009;**20**:79-83.

~~4.~~5. **Orzalesi N**, Rossetti L, Invernizzi T, et al. Effect of timolol, latanoprost, and dorzolamide on circadian IOP in glaucoma or ocular hypertension. *Invest Ophthalmol Vis Sci* 2000;**41**:2566-73.

~~5.~~6. **Liu JH**, Kripke DF, Weinreb RN. Comparison of the nocturnal effects of once-daily timolol and latanoprost on intraocular pressure. *Am J Ophthalmol* 2004;**138**:389-95.

~~6.~~7. **Sit AJ**, Weinreb RN, Crowston JG, et al. Sustained effect of travoprost on diurnal and nocturnal intraocular pressure. *Am J Ophthalmol* 2006;**141**:1131-3.

~~7.~~8. **Liu JH**, Medeiros FA, Slight JR, et al. Comparing diurnal and nocturnal effects of brinzolamide and timolol on intraocular pressure in patients receiving latanoprost monotherapy. *Ophthalmology* 2009;**116**:449-54.

~~8.~~9. **Liu JH**, Medeiros FA, Slight JR, et al. Diurnal and nocturnal effects of brimonidine monotherapy on intraocular pressure. *Ophthalmology* 2010;**117**:2075-9.

~~9.~~10. **Woodward DF**, Phelps RL, Krauss AH, et al. Bimatoprost: a novel antiglaucoma agent. *Cardiovasc Drug Rev* 2004;**22**:103-20.

~~10.~~11. **Toris CB**, Gabelt BT, Kaufman PL. Update on the mechanism of action of topical prostaglandins for intraocular pressure reduction. *Surv Ophthalmol* 2008;**53** Suppl1:S107-20.

~~11.~~12. **Williams RD**, Cohen JS, Gross RL, et al. Long-term efficacy and safety of bimatoprost for intraocular pressure lowering in glaucoma and ocular hypertension: year 4. *Br J Ophthalmol* 2008;**92**:1387-92.

~~12.~~13. **Aptel F**, Cucherat M, Denis P. Efficacy and tolerability of prostaglandin analogs: a meta-analysis of randomized controlled clinical trials. *J Glaucoma* 2008;**17**:667-73.

~~13.~~14. **Konstas AG**, Katsimbris JM, Lallos N, et al. Latanoprost 0.005% versus bimatoprost 0.03% in primary open-angle glaucoma patients. *Ophthalmology* 2005;**112**:262-6.

~~14.~~15. **Katz LJ**, Cohen JS, Batoosingh AL, et al. Twelve-month, randomized, controlled trial of bimatoprost 0.01%, 0.0125%, and 0.03% in patients with glaucoma or ocular hypertension. *Am J Ophthalmol* 2010;**149**:661-71 e1.

~~15.~~16. **Liu JH**, Zhang X, Kripke DF, et al. Twenty-four-hour intraocular pressure pattern associated with early glaucomatous changes. *Invest Ophthalmol Vis Sci* 2003;**44**:1586-90.

~~16.~~17. **Liu JH**, Kripke DF, Twa MD, et al. Twenty-four-hour pattern of intraocular pressure in the aging population. *Invest Ophthalmol Vis Sci* 1999;**40**:2912-7.

~~17.~~18. **Mansouri K**, Shaarawy T. Continuous intraocular pressure monitoring with a wireless ocular telemetry sensor: initial clinical experience in patients with open angle glaucoma. *Br J Ophthalmol* 2011;**95**:627-9.

~~18.~~19. **Liu JH**, Weinreb RN. Monitoring intraocular pressure for 24 h. *Br J Ophthalmol* 2011;**95**:599-600.

~~19.~~20. **Liu JH**, Gokhale PA, Loving RT, et al. Laboratory assessment of diurnal and nocturnal ocular perfusion pressures in humans. *J Ocul Pharmacol Ther* 2003;**19**:291-7.

~~20.~~21. **Quaranta L**, Gandolfo F, Turano R, et al. Effects of topical hypotensive drugs on circadian IOP, blood pressure, and calculated diastolic ocular perfusion pressure in patients with glaucoma. *Invest Ophthalmol Vis Sci* 2006;**47**:2917-23.

~~21.~~22. **Nakamura T**, Yamada M, Teshima M, et al. Electrophysiological characterization of tight junctional pathway of rabbit cornea treated with ophthalmic ingredients. *Biol Pharm Bull* 2007;**30**:2360-4.

~~22.~~23. **Orzalesi N**, Rossetti L, Bottoli A, et al. Comparison of the effects of latanoprost, travoprost, and bimatoprost on circadian intraocular pressure in patients with glaucoma or ocular hypertension. *Ophthalmology* 2006;**113**:239-246.

~~23.~~24. **Yildirim N**, Sahin A, Gultekin S. The effect of latanoprost, bimatoprost, and travoprost on circadian variation of intraocular pressure in patients with open-angle glaucoma. *J Glaucoma* 2008;**17**:36-9.

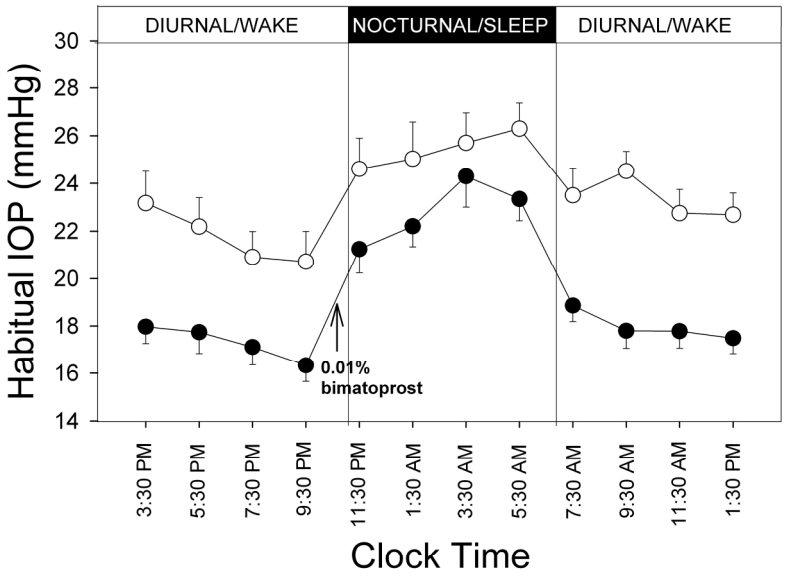
~~24.~~25. **Quaranta L**, Pizzolante T, Riva I, et al. Twenty-four-hour intraocular pressure and blood pressure levels with bimatoprost versus latanoprost in patients with normal-tension glaucoma. *Br J Ophthalmol* 2008;**92**:1227-31.

~~25.~~26. **Konstas AG**, Hollo G, Mikropoulos D, et al. Twenty-four-hour intraocular pressure control with bimatoprost and the bimatoprost/timolol fixed combination administered in the morning, or evening in exfoliative glaucoma. *Br J Ophthalmol* 2010;**94**:209-13.

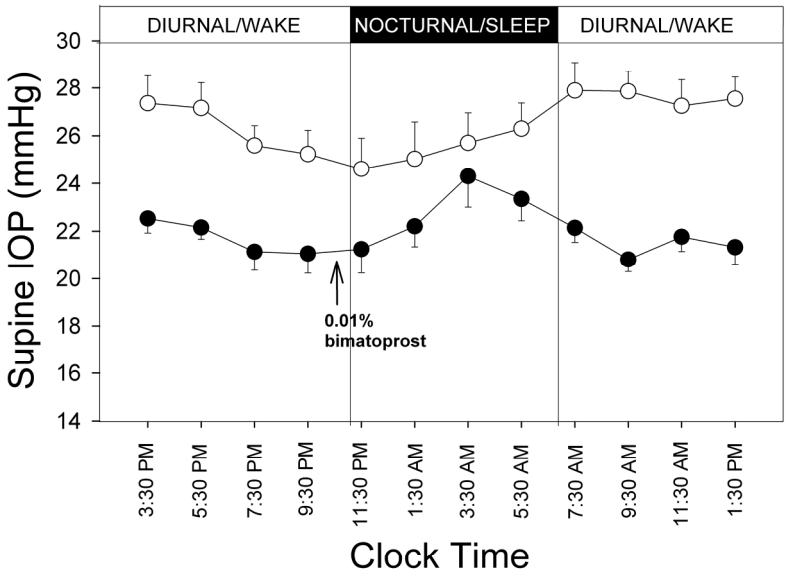
FIGURE LEGENDS

Figure 1. Profiles of 24-hour IOP in the habitual body positions. Measurements were taken from 16 subjects sitting during the diurnal period and supine during the nocturnal period. Open circles represent the baseline, and solid circles represent the bimatoprost 0.01% treatment for 4 weeks. Error bars represent standard error of the mean. IOP = intraocular pressure.

Figure 2. Profiles of 24-hour supine IOP. Open circles represent the baseline, and solid circles represent the bimatoprost 0.01% treatment for 4 weeks. Data were from the same 16 subjects as in Figure 1. Error bars represent standard error of the mean. IOP = intraocular pressure.



215x166mm (300 x 300 DPI)



215x166mm (300 x 300 DPI)