Effects of latanoprost and pilocarpine combination on the intraocular pressure and pupil size of normal rabbits

Sarchahi, A. A.[1*]; Gholipour, M. A.[2] and Toghraie, F. S.[2]

[1]Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran; [2]Graduated from School of Veterinary Medicine, Shiraz University, Shiraz, Iran

[*]**Correspondence:** A. A. Sarchahi, Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran. E-mail: sarchahi@shirazu.ac.ir

(Received 28 Sept 2010; revised version 15 Mar 2011; accepted 12 Apr 2011)

Summary

The aim of this study was to determine the combination effect of latanoprost and pilocarpine on the intraocular pressure and pupil size in normal rabbits. In this study, 18 rabbits were randomized to three groups of 6 animals each. The right eyes of rabbits in group 1 were treated topically with latanoprost, in group 2 with pilocarpine and in group 3 with latanoprost and pilocarpine. The left eyes received placebo. Drugs were instilled once a day at 8 am over 4 days. IOP and pupil diameter measurements were made at 8 am, 10 am, 12 noon, 2 pm and 4 pm during the 4 days of treatment, the 2 days that preceded treatment, and 3 days following treatment. The occurrence of blepharospasm and conjunctival hyperemia were also evaluated at the same times that the measurements were made. The mean IOPs were significantly lower than the contralateral eyes in 8 of the 20 time intervals (40%) in both latanoprost and pilocarpine-treated and in 18 of 20 time intervals (90%) in latanoprost plus pilocarpine-treated eyes in the treatment period. The mean daily hypotensive effects of latanoprost, pilocarpine and their combination were 4.5 (31%), 2 (14.4%) and 5 mmHg (34.7%), respectively. Although the mean IOPs in group 3 have decreased more than other groups, the differences between the three groups are not significant. Conjunctival hyperemia was observed in the treated eyes of the three groups. It is concluded that topical instillation of the combination of latanoprost and pilocarpine was not as effective in IOP reduction than by drugs alone and that hyperemia is the most frequent side effect observed during the treatment period.

Key words: Intraocular pressure, Latanoprost, Pilocarpine, Pupil diameter, Rabbit

Introduction

Glaucoma is the leading cause of irreversible blindness in the world (Weinreb and Khaw, 2004). Elevated intraocular pressure (IOP) is the major risk factor for the development of glaucoma and reducing IOP to a normal level is the primary goal of treatments for glaucoma and ocular hypertension (The AGIS Investigators, 2000). Latanoprost is a prostaglandin analog and a prostaglandin F (FP) receptor agonist that acts as an ocular hypotensive agent. Despite extensive research, controversies remain regarding the mechanism of action and relative clinical efficacy of the PGs (Eisenberg *et al*., 2002; Parrish *et al*., 2003; Orzalesi *et al*., 2006). Latanoprost increase aqueous humor outflow, either by enhancing the pressure-sensitive (presumed trabecular or conventional) outflow pathway or by increasing the pressure-insensitive (uveoscleral) outflow (Lim *et al*., 2008; Toris *et al*., 2008). Pilocarpine, a cholinergic agonist, in human eyes reduces intraocular pressure by stimulating postsynaptic muscarinic receptors in the ciliary muscle causing it to contract. This opens up the fluid channels in the trabecular meshwork, thus increasing trabecular outflow facility (Kaufman and Gabelt, 1997). Despite this, its effects in rabbit eyes is not decisively clear. In monkey eyes pilocarpine partially inhibited the reduction in intraocular pressure with topical prostaglandin F2a (Crawford and Kaufman, 1987; Millar and Kaufman, 1995), however, there are clinical reports that show the two drugs appeared to be additive (Fristrom and Nilsson, 1993; Toris *et al*., 2001). To the best of our knowledge there are no experimental reports regarding the combination effects of these

drugs on IOP. Thus the purpose of the study reported here was to determine the combination effect of pilocarpine and latanoprost on the intraocular pressure and pupil size in normal rabbits.

Patients and Methods

This study was approved by the Research Animal Care and Use Committee of the School of Veterinary Medicine, Shiraz University and complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. In this study 18 New Zealand white rabbits, from both sexes (9 females, 9 males) were used. Rabbits weighed (mean±SD) 2.41 ± 0.26 kg. All rabbits were frequently handled prior to the study to ensure that they were adjusted to physical manipulation. They were housed in the same laboratory facility under cyclic illumination (12 h on, 12 h off) and all measurements were performed in the same room under consistent lighting and examination conditions. All eyes were determined to be free of clinically relevant abnormalities by ophthalmic examinations. The rabbits were randomized into three groups of 6 animals each. The right eye was elected to receive treatment, while the left eye received placebo (Normal saline). Animals in group 1 were treated topically with one drop of NaCl and one drop of 0.005% latanoprost (Xalatan™, Pfizer Manufacturing Belgium NV, Puurs, Belgium), in group 2 with one drop of NaCl and one drop of 2% pilocarpine (Glaupin® 2, Sina Darou, Tehran, Iran) and in group 3 with one drop of 0.005% latanoprost and one drop of 2% pilocarpine. The drops were instilled with a 5 min interval in each eye. The experiment was divided into three consecutive periods of 2, 4, and 3 days. During the first period, IOPs in both eyes were measured under no medication for the determination of a baseline. During the second period, the right eye of each animal received a drug, whereas the left eye received placebo. Instillations were always made at 8 am. During the third period, the drugs were discontinued, and IOPs were measured in order to evaluate recovery. IOP measurements were made at 8 am, 10 am, 12 noon, 2 pm and 4 pm by the same observer. All measurements were made by a person who was unaware which treatment had been administered. All rabbits were positioned on their sternums, with their heads maintained in a normal, upright position during the measurements. Eyelids were minimally manipulated, avoiding pressure on the globe. The cornea was topically anesthetized, using 1 drop of 0.5% tetracaine (Anestocaine, Sina darou, Tehran, Iran). Intraocular pressure was measured with an applanation tonometer (Tonopen VET, Reichert Inc., NY, USA) that was used and maintained in accordance with the manufacturer's specifications. The eyes were photographed and pictures were transferred to computer and then the pupil diameters (PD) were measured by AutoCAD 2005 software. The occurrence of blepharospasm and conjunctival hyperemia were also evaluated at the same time the measurements were made.

Statistical analysis

Drug comparisons were performed using SPSS software utilizing Tukey's HSD and ANOVA tests for repeated measurements. The comparison between the IOP and PD measurements obtained in the three treatment groups at each time interval was performed using the one-way analysis of variance (ANOVA) with Tukey's HSD significant difference test for multiple comparisons. The paired Student's t-test was employed to compare the IOPs and PDs of the treated eyes to contralateral eyes that received placebo. A p-value of less than 0.02 was considered statistically significant. All results were expressed as mean ± SD.

Results

The comparison between the mean IOPs of the treated and control eyes of latanoprost, pilocarpine and latanoprost plus pilocarpine groups throughout the study are displayed in Figs. 1, 2 and 3, respectively. The mean IOPs were significantly lower than the contralateral eyes in 8 of the 20 time intervals (40%) in the latanoprost-treated eyes ($P<0.01$) and in 8 of the 20 time intervals (40%) in the pilocarpine group

(P<0.007) in the treatment period, whereas mean IOPs in the latanoprost plus pilocarpine combination group were lower in 18 of 20 time intervals (90%) in the treatment period compared to contralateral eyes (P<0.006).

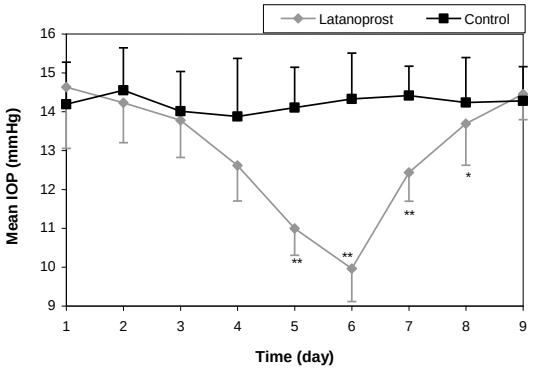

Fig. 1: IOPs in latanoprost treated and control eyes throughout the study. Data are expressed as the mean±SD of six rabbits. *P<0.05 and **P<0.01

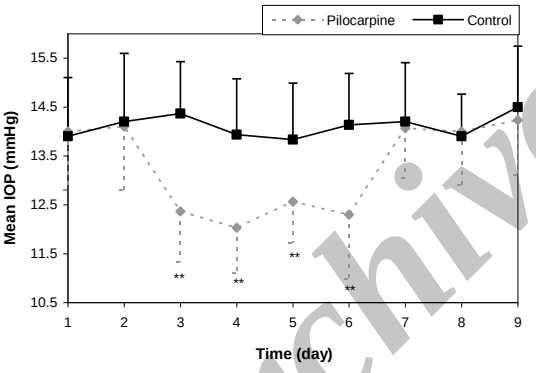

Fig. 2: IOPs in pilocarpine treated and control eyes throughout the study. Data are expressed as the mean±SD of six rabbits. **P<0.01

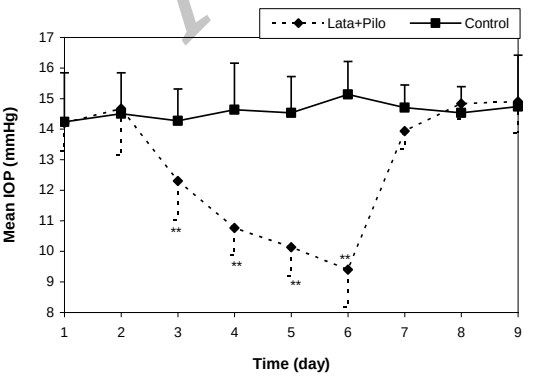

Fig. 3: IOPs in latanoprost-pilocarpine combination treated and control eyes throughout the study. Data are expressed as the mean±SD of six rabbits. **P<0.01

Repeated measure analysis of IOPs in the latanoprost group showed that there was a significant decrease in IOP over time (P=0.002); and interaction effects of time by eye showed significant decreases in treated eyes compared to placebo eyes (P=0.003). The IOP was starting to decrease on day 3 and reached a maximum on day 6. During the third period (recovery), there was a gradual increase of IOP back to baseline values (Fig. 1). The maximum ocular hypotensive effect caused by latanoprost (IOP reduction of 6.6 mmHg (45.3%) from baseline occurred on the sixth day of treatment at 2 pm and the mean daily hypotensive effect of this drug was 4.5 mmHg (31%), that occurred on the sixth day of treatment (One way ANOVA results). On diurnal IOP, latanoprost caused an early increase of IOP at 10 am (2 h after instillation) and then IOP decreased until 2 pm (6 h after instillation). This effect was not seen by the latanoprost plus pilocarpine combination group (Fig. 4).

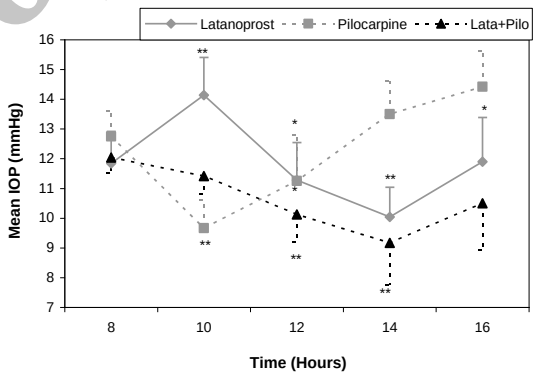

Fig. 4: Diurnal IOP changes in the right (treated) eyes of the three groups during the treatment period (n=6). Data are expressed as the mean±SD of six rabbits. *P<0.05 and **P<0.01 *vs.* IOP change in baseline values (8 am) of each drug

In the pilocarpine group, IOPs significantly decreased over time (P=0.005); and interaction effects of time by eye showed significant decreases in treated eyes compared to placebo eyes (P=0.008, repeated measure ANOVA). The IOP mostly decreased between days 3 to 6 (Fig. 2) (repeated measure analysis), then the IOPs increased to baseline values on the recovery period. The maximum hypotensive effect was seen on the fourth day of

treatment at 10 am and was 4.8 mmHg (33.8%), and the mean daily hypotensive effect of this drug was 2 mmHg (14.4%) occurring on the fourth treatment day (One way ANOVA results).

In the latanoprost plus pilocarpine combination group, the IOPs significantly decreased during the study (P=0.001); and time by eye interactions showed that the combination of the two drugs significantly decreased the IOP in treated eyes compared to placebo eyes (P<0.001) (repeated measure analysis). The IOP started to decrease on day 3 and reached a maximum on day 6 (Fig. 3), increasing to normal baseline values on day 7. The maximum hypotensive effect of this drug combination (6.2 mmHg (43.2%) from the baseline) occurred at 2 pm on the sixth day of the treatment period and the mean daily reduction of IOP by this group was 5 mmHg (34.7%), seen on day 6 (one way ANOVA results).

There was no statistically significant difference between the mean IOPs of eyes treated with latanoprost, pilocarpine and the combination groups throughout the study (Fig. 5) (P>0.05, repeated measure ANOVA). However, the IOPs in 7 out of 20 time intervals (35%) in the treatment period in the latanoprost group was significantly lower than the pilocarpine group; and in 9 out of 20 time intervals (45%) the IOPs in the combination group was significantly lower than the pilocarpine group and in 3 of 20 time intervals (15%) in the latanoprost plus pilocarpine combination group was lower than the latanoprost group (P<0.02, one way ANOVA). These results show that pilocarpine did not block or attenuate the uveoscleral outflow effect of latanoprost (Fig. 5). For left eyes, the comparison between the three groups also revealed no significant differences (P>0.05). The IOPs in the latanoprost group after discontinuing the drug started to return to normal ranges and reached normal baseline values on day 9, but in the pilocarpine and latanoprost plus pilocarpine combination groups it reached normal values on day 7.

Although pupil diameters decreased by the three groups in some time intervals during the treatment period, the results of repeated measure ANOVA showed no significant differences between the three groups in neither the treated nor the placebo eyes (P>0.05).

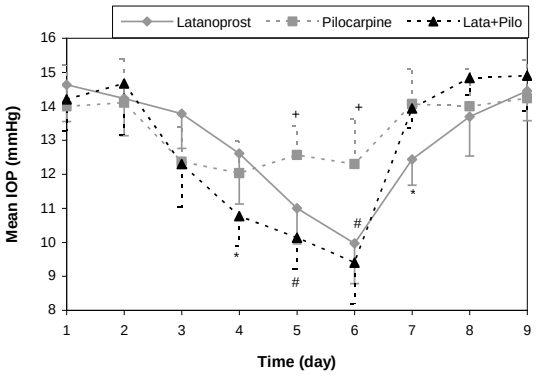

Fig. 5: IOPs in right (treated) eyes of three groups throughout the study (n=6). Data are expressed as the mean±SD of six rabbits. *Between latanoprost and Lata+Pilo, P<0.05. +Between latanoprost and pilocarpine, P<0.05. #Between pilocarpine and Lata+Pilo, P<0.01

Conjunctival hyperemia was identified in the treated eyes of the three groups during the entire treatment period several minutes after instillation of drugs at 8 am and gradually decreased until 12 pm. There was no hyperemia at 2 pm.

Discussion

In the present study, the mean daily IOP reduction from the baseline was 4.5 mmHg (31%) for latanoprost, 2 mmHg (14.4%) for pilocarpine and 5 mmHg (34.7%) for their combination. On the other hand, the peak effects of latanoprost, pilocarpine and their combination were 45.3, 33.8 and 43.2%, respectively; thus our findings indicate that the three groups reduced the IOP during the treatment period; However, there was no statistically significant difference between the mean IOP reductions of the eyes treated in the three groups. The findings of our study are in accordance with those observed by Pintor *et al*. (2004) and Gupta *et al*. (2007) in normotensive rabbits after single-drop application of latanoprost 0.005%; however, the peak effect observed by Pintor *et al*. (2004) (33.14%) and Gupta *et al*. (2007) (22.56%) was less than that in our study. The reason for the difference in observation may be attributed to the method of IOP estimations as Gupta *et al*. (2007) measured IOP using the noncontact tonometer. Other explanations for the

apparent excess efficacy of latanoprost and pilocarpine in the present study include the higher baseline intraocular pressures in our study than those in the earlier studies (Hayashi *et al*., 1989); as Gupta *et al*. (2007) reported a 40.24% and 28.91% reduction of IOP by latanoprost and pilocarpine, respectively, in hypertensive rabbits. In other studies, the latanoprost was found to have no effect on normal rabbit IOP (Ishii *et al*., 2001; Orihashi *et al*., 2005). The difference in the observations from these studies can again be attributed to the difference in the methodology used. Ishii *et al*. (2001) did the measurements of rabbit IOP under general anesthesia, and Orihashi *et al*. (2005) measured IOP using the pneumatonograph, whereas in our study IOP estimations were done in conscious rabbits using a Tonovet. Another reason for the efficacy of latanoprost in the present study includes differences of time of IOP estimation after drug instillation. They measured the IOP immediately after instillation until maximally 90 min (Ishii *et al*., 2001) or 240 min later (Orihashi *et al*., 2005). As our results show, when latanoprost is used alone, IOP increased until 2 h after instillation and then decreased to a minimum point 6 h after instillation. Interestingly, in the present study pilocarpine prevented this early latanoprost-related increase of IOP (Fig. 4).

In the present study IOP reduction by latanoprost was more than pilocarpine and as shown in Figs. 1 and 5, the IOP gradually reduced and reached a maximum on day 6. Our findings are in accordance with those observed by Gupta *et al*. (2007), as they reported a higher peak reduction of latanoprost (22.56%) compared to pilocarpine (18.23%) on rabbit IOP.

Among the most frequent side effects observed with the use of latanoprost in human beings are conjunctival hyperemia, iris pigmentation (Rowe *et al*., 1997), eyelash changes, and superficial punctate epithelial erosions (Lass *et al*., 2001). Cases of iritis and anterior uveitis have also been described (Moroi *et al*., 1999).

In our study, conjunctival hyperemia was the most common side-effect in the three groups. Hyperemia was observed immediately after drug instillations at 8 am and gradually decreased to minimum at 12 noon. There was no hyperemia at 2 pm. Localized vessel dilation associated with prostaglandins is thought to be related to the release of the ubiquitous vasodilator, nitric oxide. However, the mechanism by which the release of nitric oxide occurs is not known exactly (Resul and Stjernschantz, 1993; Stewart *et al*., 2003). Some studies have suggested that latanoprost may cause significantly less short-term conjunctival hyperemia on average than other prostaglandins in healthy subjects (Sherwood and Brandet, 2001; Woodward *et al*., 2001; Stewart *et al*., 2003).

In our study, pupil diameters in some time intervals in the treated eyes of the three groups decreased compared to placebo eyes; in spite of this, there were no significant differences between treated and control eyes of the three groups (repeated measure analysis). Gupta *et al*. (2007) also, reported no pupillary constriction by latanoprost treatment in rabbits.

We conclude that 1) topical instillation of a combination of latanoprost 0.005% and pilocarpine 2% is not more effective in reducing the IOP than by drugs alone in normal rabbits; however our findings indicate that pilocarpine did not prevent the IOP-lowering effect of latanoprost; and that 2) hyperemia is the most frequent side effect observed during the treatment period; however, it is important to emphasize that the treatment period was short in our study, and prolonged use of prostaglandin analogs may lead to the development of other side effects.

References

Crawford, KS and Kaufman, PL (1987). Pilocarpine antagonizes prostaglandin F2a-induced ocular hypotension in monkeys. Evidence for enhancement of uveoscleral outflow by prostaglandin F2a. Arch. Ophthalmol., 105: 1112-1116.

Eisenberg, DL; Toris, CB and Camras, CB (2002). Bimatoprost and travoprost: a review of recent studies of two new glaucoma drugs. Surv. Ophthalmol., (Suppl.), 47: 105-115.

Fristrom, B and Nilsson, SEG (1993). Interaction of PhXA41, a new prostaglandin analogue, with pilocarpine. A study on patients with elevated intraocular pressure. Arch.

Ophthalmol., 111: 662-665.

Gupta, SK; Agarwal, R; Galpalli, ND; Srivastava, S; Agrawal, SS and Saxena, R (2007). Comparative efficacy of pilocarpine, timolol and latanoprost in experimental models of glaucoma. Methods Find. Exp. Clin. Pharmacol., 29: 665-671.

Hayashi, M; Yablonski, ME and Novack, GD (1989). Trabecular outflow facility determined by fluorophotometry in human subjects. Exp. Eye Res., 48: 621-625.

Ishii, K; Tomidokoro, A; Nagahara, M; Tamaki, Y; Kanno, M; Fukaya, Y and Araie, M (2001). Effects of topical latanoprost on optic nerve head circulation in rabbits, monkeys, and humans. Invest. Ophthalmol. Vis. Sci., 42: 2957-2963.

Kaufman, PL and Gabelt, BT (1997). Direct, indirect, and dual-action parasympathetic drugs. In: Zimmerman, TJ; Kooner, KS; Sharir, M and Fechtner, RD (Eds.), *Textbook of ocular pharmacology*. (3rd Edn.), Philadelphia, Lippincott: Williams and Wilkins. PP: 221-238.

Lass, JH; Eriksson, GL; Osterling, L and Simpson, CV (2001). Comparison of the corneal effects of latanoprost, fixed combination latanoprost-timolol, and timolol: a double-masked, randomized, one-year study. Ophthalmology. 108: 264-271.

Lim, KS; Nau, CB; O'Byrne, MM; Hodge, DO; Toris, CB; McLaren, JW and Johnson, DH (2008). Mechanism of action of bimatoprost, latanoprost, and travoprost in healthy subjects. A crossover study. Ophthalmology. 115: 790-795.e4.

Millar, JC and Kaufman, PL (1995). PGF2a/pilocarpine interactions on IOP and accommodation in monkeys. Exp. Eye Res., 61: 677-683.

Moroi, SE; Gottfredsdottir, MS; Schteingart, MT; Elner, SG; Lee, CM; Schertzer, RM; Abrams, GW and Johnson, MW (1999). Cystoid macular edema associated with latanoprost therapy in a case series of patients with glaucoma and ocular hypertension. Ophthalmology. 106: 1024-1029.

Orihashi, M; Shima, Y; Tsuneki, H and Kimur, I (2005). Potent reduction of intraocular pressure by nipradilol plus latanoprost in ocular hypertensive rabbits. Biol. Pharm. Bull., 28: 65-68.

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

Parrish, RK; Palmberg, P; Sheu, WP and XLT Study Group (2003). A comparison of latanoprost, bimatoprost, and travoprost in patients with elevated intraocular pressure: a 12-week, randomized, masked-evaluator multicenter study. Am. J. Ophthalmol., 135: 688-703.

Pintor, J; Peláez, T and Peral, A (2004). Adenosine tetraphosphate, AP4, a physiological regulator of intraocular pressure in normotensive rabbit eyes. J. Pharmacol. Exp. Ther., 308: 468-473.

Resul, B and Stjernschantz, J (1993). Structure-activity relationships of prostaglandin analogues as ocular hypotensive agent. Expert. Opin. Ther. Pat., 3: 781-795.

Rowe, JA; Hattenhauer, MG and Herman, DC (1997). Adverse side effects associated with latanoprost. Am. J. Ophthalmol., 124: 683-685.

Sherwood, M and Brandet, J (2001). Six-month comparison of bimatoprost once daily and twice daily with timolol twice daily in patients with elevated IOP. Surv. Ophthalmol., 45: 361-368.

Stewart, WC; Kolker, AE; Stewart, JA; Leech, J and Jackson, AL (2003). Conjunctival hyperemia in healthy subjects after short-term dosing with latanoprost, bimatoprost, and travoprost. Am. J. Ophthalmol., 135: 314-320.

The AGIS Investigators (2000). The advanced glaucoma intervention study (AGIS): 7. The relationship between control of intraocular pressure and visual field deterioration. Am. J. Ophthalmol., 130: 429-440.

Toris, CB; Gabelt, BT and Kaufman, PL (2008). Update on the mechanism of action of topical prostaglandins for intraocular pressure reduction. Surv. Ophthalmol., (Suppl. 1), 53: 107-120.

Toris, CB; Zhan, GL; Zhao, J; Camras, CB and Yablonski, ME (2001). Potential mechanism for the additivity of pilocarpine and latanoprost. Am. J. Ophthalmol., 131: 722-728.

Weinreb, RN and Khaw, PT (2004). Primary open-angle glaucoma. Lancet. 363: 1711-1720.

Woodward, DF; Krauss, AH; Chen, J; Lai, RK; Spada, CS; Burk, RM; Andrews, SW; Shi, L; Liang, Y; Kedzie, KM; Chen, R; Gil, DW; Kharlamb, A; Archeampong, A; Ling, J; Madhu, C; Ni, J; Rix, P; Usansky, J; Usansky, H; Weber, A; Welty, D; Yang, W; Tang-Liu, DD; Garst, ME; Brar, B; Wheeler, LA and Kaplan, LJ (2001). The pharmacology of bimatoprost (Lumigan). Surv. Ophthalmol., 45: 337-345.