Effects of concomitant administration of latanoprost and pilocarpine on the intraocular pressure of experimentally glaucomatous rabbits

Bozorgi, H.[1] **and Sarchahi, A. A.**[2*]

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

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Summary

The purpose of this study was to evaluate the effects of a combination of latanoprost and pilocarpine on the intraocular pressure in experimentally glaucomatous rabbits. Ocular hypertension was induced in 11 conscious rabbits by the oral administration of tap water (70 mL/kg) via an orogastric tube. The study was conducted in three therapeutic groups to test the effects of latanoprost (group L), pilocarpine (group P) and a combination of the two drugs (group LP). After evaluating the first drug, a washout period of 1 week was allowed before the second drug was evaluated. The left eye of rabbits in group L received one drop of normal saline and one drop of 0.005% latanoprost, in group P one drop of normal saline and one drop of 2% pilocarpine and in group LP, one drop of 0.005% latanoprost and one drop of 2% pilocarpine. The right eyes of all rabbits received two drops of normal saline (as control eyes). The intraocular pressure (IOP) and pupil diameter (PD) were measured before and at 0, 15, 30, 45, 60, 75, 90, 105 and 120 min post-water loading. The results showed that eyes, which received pilocarpine had a significantly lower increase in IOP at 15, 30, 45 and 60 min after drug administration compared to placebo eyes, whereas groups L and LP did not prevent an IOP increase. The PD decreased in groups P and LP throughout the study, whereas it did not change in group L. It was concluded that the topical administration of latanoprost alone or in combination with pilocarpine appeared to have no effect on IOP in acute ocular hypertensive rabbits.

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

Introduction

Glaucoma is a disease that is characterized by progressive optic nerve head cupping and loss of the visual field (Kerrigan-Baumrind *et al*., 2000; Harwerth *et al*., 2002). Specific visual field defects, due to the loss of retinal ganglion cells and damage to the optic nerve head, frequently lead to blindness (Baltmr *et al*., 2010). The major risk factor for most cases of glaucoma is high intraocular pressure (IOP) (Mao *et al*., 1991; Martinez-Bello *et al*., 2000). Therefore, antiglaucoma therapy primarily aims to control the IOP. The medical management of glaucoma involves the use of drugs, individually or in combination, that act by various mechanisms such as cholinergics, β-blockers, α-agonists, carbonic anhydrase inhibitors and prostaglandin analogs (Gelatt, 2007). Combination therapy for the reduction of IOP is widely used nowadays because more than 50% of human patients require additional therapy to maintain long-term healthy IOPs (Kass *et al*., 2002). There are several controversies regarding the effects of latanoprost in rabbits. Pintor *et al*. (2004) and Gupta *et al*. (2007) showed that latanoprost significantly decreases the IOP in normotensive and experimentally induced hypertensive rabbits after single-drop applications of the drug. However, in other studies, latanoprost was found to have no effect on IOP in normal or hypertensive rabbits (Ishii *et al*., 2001; Orihashi *et al*., 2005). We have already shown that latanoprost significantly decreases the IOP in normotensive rabbits (Sarchahi *et al*., 2011). We also showed that latanoprost did

not prevent the IOP-lowering effect of pilocarpine in rabbits, and that although the combined effect of these two drugs was slightly increased; it was not significant (Sarchahi *et al*., 2011). We assumed that the primary IOP of normal rabbits was so low that the IOP reduction due to the combination of latanoprost and pilocarpine was not demonstrable. Thus, it was proposed that a combination of these two drugs would reduce the IOP significantly more than each drug alone in hypertensive rabbits in which the primary IOP was high enough. Thus, the purpose of this study was to evaluate the effect of a combination of latanoprost and pilocarpine in experimental ocular hypertension in rabbits.

Materials 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. Eleven New Zealand white rabbits of both sexes (five males and six females), weighing 1-3 kg (mean ± SD, 1.77 ± 0.53 kg), were maintained for one week in a standard room on a 12-h light-dark schedule in order for them to get used to the environment. Food and water were provided *ad libitum*. All eyes were found to be free of clinically relevant abnormalities by ophthalmic examination, including slit-lamp bio-microscopy, ophthalmoscopy and tonometry. The rabbits were trained for one week to accept tonometry and IOP was measured daily during this time. Once the rabbits had been trained to accept tonometry, IOPs were estimated over 24 h to determine the diurnal variations of IOP in normal rabbit. All rabbits were used to study the effects of three therapeutic groups.

The animals were fasted overnight and the basal IOPs were estimated. The rabbits were subjected to baseline IOP estimations three times with intervals of 15 min, starting at 8.30 to 9.00 a.m. The average of three estimates was taken as the baseline value in normotensive rabbits for each of the three drugs. In order to test the drugs, the left eyes of rabbits in group L received one drop of normal saline and one drop of 0.005% latanoprost (Xalatan^{TM}, Pfizer Manufacturing Belgium NV, Puurs, Belgium), group P one drop of normal saline and one drop of 2% pilocarpine (Glaupin^{R} 2, Sina Darou, Tehran, Iran), and group LP one drop of 0.005% latanoprost and one drop of 2% pilocarpine. All drops were instilled with 5 min intervals. The right eyes of all rabbits received two drops of normal saline as the placebo. Ocular hypertension was induced in conscious rabbits by the oral administration of tap water (70 mL/kg) via an orogastric tube. The IOPs were measured at 0, 15, 30, 45, 60, 75, 90, 105 and 120 min after water loading using an applanation tonometer (Tonopen VET, Reichert Inc., Depew, NY, USA), which was used according to the manufacturer's instructions. The eyes were photographed immediately after IOP measurements using a digital camera (Cannon PowerShot A630, Hongkong, China), and the photos were downloaded to a computer in order to determine the pupil diameter (PD) using AutoCAD 2005 software. After a washout period of one week following the evaluation of one drug, the second drug was tested. Pilocarpine and latanoprost were instilled 15 min and 3.4 h before water loading, respectively. The presence of blepharospasm and conjunctival hyperemia was objectively determined (at the same time as the IOP and PD measurements) by the same examiner, and the rates were scored for each eye (0 = none, 1 = mild, 2 = moderate, 3 = severe).

Statistical analysis

The Wilcoxon signed ranks test was used to compare the IOP of the treated eyes with the baseline and placebo values in all three treatments. A p-value of less than 0.05 was considered statistically significant. All results are expressed as mean ± SD.

Results

Diurnal variation

Figure 1 shows the changes in IOPs of healthy rabbits over 24 h. The diurnal IOP (average of left and right eyes) varied between 13.5 mmHg (maximum) at 9 a.m.

and 9.2 mmHg (minimum) at 10 p.m. There were no significant differences (P≥0.05) in the IOP between left and right eyes at any time point.

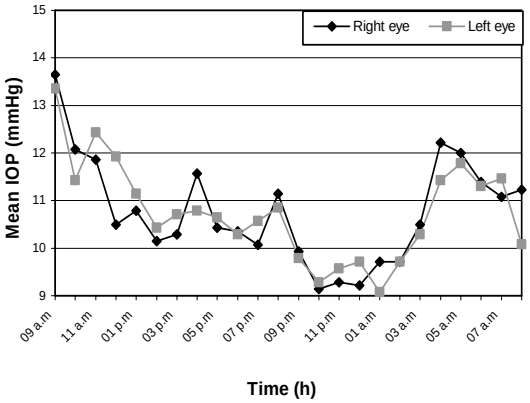

Fig. 1: Diurnal variation in IOPs in normotensive rabbits. The maximum IOP was recorded at 9 a.m. and the minimum IOP was recorded at 10 p.m. No significant differences were observed between the left and right eyes at any time point

Effect of 0.005% latanoprost on IOP

The baseline IOPs of the left and right eyes in group L were 12.8 ± 2.6 and 12.4 ± 1.9 mmHg, respectively. There was no significant difference (P≥0.05) between left and right eyes in the baseline values. Fifteen minutes after water loading, the IOP increased in both treated and placebo eyes and reached to 21.9 ± 8.1 (71.1%, p=0.004) and 22.7 ± 9.4 (83.1%, p=0.008) mmHg, respectively. The IOP reached its highest level after 30 min (23 ± 8 mmHg, 79.7% increase in treated eye and 24 ± 10.8 mmHg, 93.5% increase in placebo eye) and then started to decline, although it was significantly higher than the baseline values until 105 min after water loading. There were no significant differences (P≥0.05) between the treated and placebo eyes throughout the procedure. Thus, latanoprost appears not to be effective in the prevention of IOP increase (Fig. 2). The pupil diameters did not change after water loading in the treated eyes when compared to both pre-baseline values and placebo eye values (P>0.05).

IOP-lowering effect of 2% pilocarpine

The baseline IOPs in the treated and

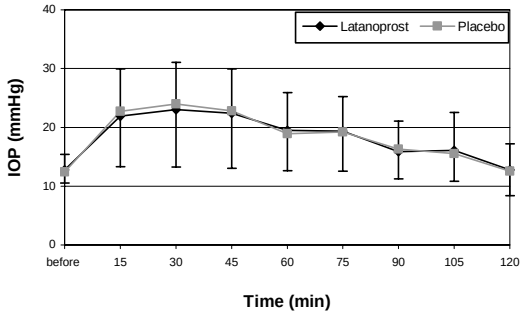

Fig. 2: Effect of latanoprost on the IOP in experimentally induced ocular hypertensive rabbits. The IOPs significantly increased 15 min after water loading in both eyes. The IOP reached its highest level after 30 min. It was significantly higher than the baseline values until minute 105. There were no differences between the two eyes. Data are expressed as the mean ± SD of 11 rabbits

placebo eyes in group P were 16.7 ± 3.1 and 16 ± 2.7 mmHg, respectively. There were no differences (P≥0.05) between the two eyes regarding the baseline values. In this group, the IOPs also increased 15 min after water loading and reached 20.5 ± 7.1 mmHg (22.8%) (P=0.21) and 26.1 ± 10.3 mmHg (61.1%) (P=0.02) in the treated and placebo eyes, respectively. The IOP reached its maximum point after 30 min (25.5 ± 6.8 mmHg, 52.7% increase in treated eyes, and 29.5 ± 8.8 mmHg, 82.1% increase in placebo eyes) and then started to decline, although it was significantly (P<0.05) higher than the baseline values until 60 and 90 min after water loading in the treated and placebo eyes, respectively. In the treated eyes, pilocarpine provided significant protection against the rise in IOP induced by water loading, which started from minute 15 with a difference of 5.6 mmHg (21.4%) from the placebo and continued until minute 60. The maximum difference between the IOP in treated and placebo eyes was 21.4% at 15 min after water loading (Fig. 3).

The pupil diameters also significantly decreased immediately after pilocarpine instillation, which lasted for 2 h (end of the study).

Effect of 0.005% latanoprost plus 2% pilocarpine on IOP

As with the other two groups, 15 min after water loading the IOPs in the treated and placebo eyes also increased in LP

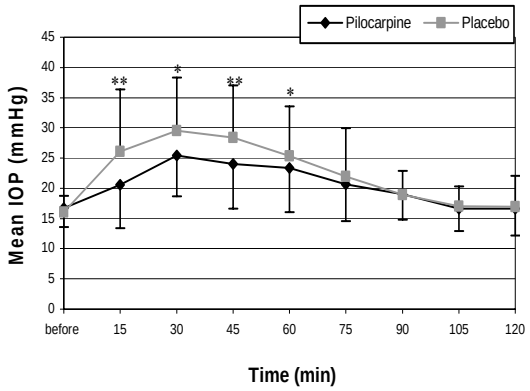

Fig. 3: Effect of pilocarpine on the IOP in experimentally induced ocular hypertensive rabbits. The IOPs significantly increased 15 min after water loading in both eyes. The IOP reached its maximum point after 30 min. It was significantly higher than the baseline values until 60 and 90 min after water loading in the treated and placebo eyes, respectively. Data are expressed as the mean ± SD of 11 rabbits. Pilocarpine prevented an increase in IOP in the treated eyes from minutes 15 to 60. * P<0.05, and ** P<0.01

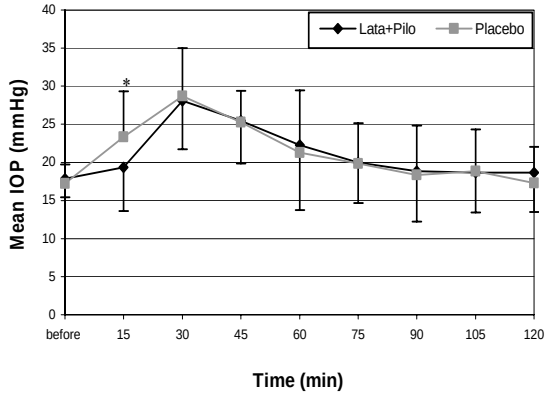

Fig. 4: Effect of the combination of latanoprost and pilocarpine on the IOP in experimentally induced ocular hypertensive rabbits. The IOPs significantly increased 15 min after water loading in both eyes. The IOP reached its maximum level after 30 min. The rise in IOP remained significant until minute 45. The IOP in treated eyes was only significantly lower than in the placebo eyes at 15 min after water loading. Data are expressed as the mean ± SD of 11 rabbits. * P<0.05

treatment very quickly from 17.8 ± 2.4 and 17.2 ± 2.5 mmHg at baseline to 19.4 ± 5.8 mmHg (9% increase, P=0.39) and 23.4 ± 6 mmHg (36% increase, P=0.01), respectively. This increase in IOP continued and reached a maximum level after 30 min (28.1 ± 6.4 mmHg, 57.9%, and 28.7 ± 6.3 mmHg, 66.9% increase in treated and placebo eyes, respectively) and then it started to decline. The rise in IOP remained significant until minute 45, after which it was overloaded with water and it was no longer different from the baseline values after 60 min. In the treated eyes, the IOP increase was the same as for the placebo eyes and similar to group L; thus, the combination of drugs did not prevent the induced increase in IOP by water loading. In this group, the IOP in the treated eyes was significantly (P<0.05) lower than in the placebo eyes only at 15 min after water loading (19.4 ± 5.8 compared to 23.4 ± 6, respectively, P=0.049) (Fig. 4).

Pupil diameters in the treated eyes in this group, as in group P, significantly decreased compared to the baseline and placebo eyes immediately after drug instillation, which lasted for 2 h (P<0.01).

Conjunctival hyperemia was observed in all three groups of the treated eyes during treatment period. The hyperemia was observed a few minutes after drug administration and reached its maximum 2 h later, and then it gradually decreased over the next 2 h. There were no differences in the level of conjunctival hyperemia between the three groups.

Discussion

The diurnal rhythm of IOP in the present study was variable. The IOP was found to be the highest and lowest at 9 a.m. and at 10 p.m, respectively. Although generally IOP seemed to decrease gradually during the day after 9 a.m. and increase during the night, there are some fluctuations in these trends. McLaren *et al*. (1996) showed that IOP rises with the onset of darkness and falls with the onset of light. They demonstrated that the IOP was highest between 2 and 4 h after the onset of darkness and remained high or gradually decreased through the night. With the onset of light, IOP dropped and remained low until the return of darkness. Intraocular pressure was found to be at its lowest level between 3 and 6 h after the onset of light (McLaren *et al*., 1996). In the present study, because the animals were handled during measurements, the diurnal cycle is probably influenced by physiologic

response to the presence of an investigator or to the handling. Another cause of IOP fluctuation in the present study compared to the previous study (McLaren *et al*., 1996) can be attributed to the effect of light during measurement of IOPs at dark periods.

In the present study, latanoprost alone or in combination with pilocarpine did not prevent the rise of intraocular pressure in rabbits. This result is in contrast to the previous reports about the effect of latanoprost on the IOP in normotensive and hypertensive rabbits (Pintor *et al*., 2004; Gupta *et al*., 2007; Sarchahi *et al*., 2011). Although the reason for these differences is not exactly clear, there are some differences in the methodology of these studies that could explain it including: 1) latanoprost was used over five consecutive days in our previous study but in the present study, latanoprost was only instilled once. 2) In our previous study, the IOPs were measured up to 8 h after drug instillation (every 2 h). Previous study also showed that latanoprost is more effective in lowering IOP after the first few days. Moreover, some other reports showed that the effects of latanoprost increase gradually. Mandić *et al*. (2002) reported that latanoprost, when used alone, decreased the intraocular pressure from 21.9 to 17.4 mmHg after 15 days. The IOP decreased to 16.7 mmHg after two months and reached 16.6 mmHg after six months. 3) Gupta *et al*. (2007) reported that the maximum effect of latanoprost on the IOP in normotensive rabbits occurs 3.4 h after instillation. In the present study, we used latanoprost 3.4 h before water loading (based on the study by Gupta *et al*. 2007), and the IOPs were measured up to 2 h after water loading. The time of the maximal effect of latanoprost in the present study may have been before the induction of hypertension or it may have been after the time of the IOP measurements. 4) The type of the study could affect the results. Gupta *et al*. (2007) reported that the maximum IOP increase occurred 60 min after water loading whereas in the present study, in all three groups, it occurred 30 min after water loading and then started to decrease. Moreover, after 105, 60-90 and 45 min (in groups L, P and LP, respectively) it returned to the baseline values. 5) Gupta *et al*. (2007) used a non-contact tonometer whereas in the present study tonopen Vet (a contact tonometer) was used. 6) The episcleral pressure probably increases to a level (Ruiz-Ederra and Verkman, 2006) where latanoprost is not able to overcome it and could not increase the uveoscleral outflow of aqueous humor, the major way by which latanoprost reduces the pressure. More studies using a longer period of hypertension and a longer use of latanoprost are needed in order to determine which of these mechanisms plays a more important role. However, several other reports show that latanoprost is not effective in IOP reduction in normotensive or hypertensive rabbits (Ishii *et al*., 2001; Orihashi *et al*., 2005). It has been shown that prostaglandin F (FP) receptors for latanoprost do not contribute to IOP reduction in rabbits (Woodward *et al*., 1989) and rabbits' eyes are resistant to latanoprost and do not easily respond to it. On the other hand, pilocarpine has been shown to have an antagonistic effect on prostaglandin-induced IOP reduction in primates (Crawford and Kaufman, 1987; Millar and Kaufman, 1995; Serle *et al*., 2001). It seems that latanoprost in combination group has prevented the pilocarpine effect as what occurs in monkeys.

It is concluded that the topical administration of latanoprost alone or in combination with pilocarpine has no effect on the IOP in acute ocular hypertension in rabbits.

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