Effect of topically applied nimodipine on the intraocular pressure on ocular normotensive and betamethasone-induced hypertensive eyes in rabbits

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ABSTRACT

Glaucoma is a leading cause of irreversible blindness worldwide. Lowering intraocular pressure (IOP) is the only strategy documented to delay the appearance and retard the progression of vision loss. The present investigation was conducted to evaluate the effect of topical nimodipine (0.5%) drop on IOP of both normotensive and induced hypertensive eyes in rabbits. Thirty-six rabbits were included in the present study. Two groups (each group contains 6 rabbits) were used to evaluate the effect of nimodipine, one as a normotensive group and the other for induction of hypertensive. Nimodipine ophthalmic solutions were instilled twice daily for 7 days in both normotensive and induced hypertensive eyes (that induced by weakly subconjunctival injection of 0.7 ml of betamethasone suspension for 4 weeks). On the other hand, another two groups were used to evaluate the effect of (twice daily installation for 7 days) of Timolol and distilled water (DW). IOP measured by Schiotz tonometer daily at about the same time (9.0 am) to avoid IOP diurnal fluctuation. The main findings of the present study were the followings: Nimodipine (0.5%) was very effective in lowering IOP in both normotensive and ocular hypertensive rabbits. Mean IOP decreased after one day of nimodipine installation by (1.5 ± 0.43 mmHg) in normotensive eyes and by (4.5 ± 1.45 mmHg) in hypertensive eyes. A significant results (P ≤ 0.05) were obtained when compared the results of nimodipine with DW (as a negative control group), on the other hand, no significant differences were obtained when compared with that of Timolol (as a positive group). The results obtained in this study provide experimental evidence for the effectiveness of nimodipine (0.5%) ophthalmic solutions in the reduction of IOP. In addition to the above nimodipine in applied doses were found to have a tolerable ocular hypotensive effect.

INTRODUCTION

The effect of calcium channels blockers (CCBs) on aqueous humor dynamics and IOP remains controversial since a wide range of results has been obtained. After systemic administration, CCBs have generally failed to reduce IOP in both rabbits (Kelly et al., 1998) and humans although several laboratories have reported ocular hypertensive and ocular hypotensive responses after oral or intravenous administration of these drugs (Waleed et al., 2015). The aim of the present work was to study the effect of nimodipine on IOP in an
animal model of both normotensive and hypertensive (betamethasone-induced ocular hypertension) in rabbits. The corticosteroid glaucoma is among experimental models more closely resembling human disease since both its clinical features (elevated IOP and gonioscopically open-angle), and underlying mechanism (reduced aqueous outflow) mimic those of human chronic open-angle glaucoma. In contrast, most of the induced experimental models for glaucoma, corticosteroid glaucoma is also observed in ophthalmological practice after topical, periocular or systemic administration of corticosteroids, a fact that strengthens the parallel between the animal and human disease (Santafe et al., 1997). Furthermore, evidence suggesting that endogenous glucocorticoids may play a role in the development of ocular hypertension in humans seems to support the utility of this glaucoma model (Mohammed et al., 2017).

**MATERIALS AND METHODS**

**Experimental animals**

Thirty-six male albino rabbits weighing 1.5-2.5 kg with no signs of ocular inflammation or gross abnormality were used in this study. The animals allocated into 2 groups represent the ocular normotensive and induced hypertensive groups. Each group further subdivided into 3 subgroups with 6 animals in each subgroup. Animals in one subgroup receive nimodipine while the other 2 subgroups receive Timolol and distilled water (DW), respectively. Animals were housed individually in plastic cages; all rabbits were maintained conventionally during the study with regulated air temperature (15-21 °C), artificial light cycle (12 hours light/12 hours’ darkness) and good ventilation. They fed a standard rabbit diet and had free access to drinking water. Animal experiments conformed to the ARVO Statement for the Use of Animals in Research and approved by the Al-Nahrain University Graduate and Ethical Committee.

**Experimental technique**

After local anesthetization of the cornea with 1-2 drops of 4% lidocaine HCL drop, the animal was held on his back, and Schiotz tonometer is placed on the cornea. A control or zero time value of IOP was taken 15 minutes before the administration of nimodipine. One drop of freshly prepared nimodipine was instilled in the middle of inferior conjunctival sac followed by lid closure. Thereafter, IOP was measured after 1 hour of topical application of the tested drug. Tested drug instilled as one drop (50μl) twice daily for 7 days and IOP measured daily at about the same time (9.0 am) to avoid diurnal IOP fluctuation. Left eye of the rabbits was used for evaluation of the tested drugs. On the other hand, right eye were considered as a control in which DW was instilled for comparison with the left eye if there is the contralateral effect of nimodipine or any side effect, change in pupil diameter and conjunctival redness of topical application of nimodipine.

**Effect of Nimodipine, Timolol and DW on Ocular Normotensive Rabbits**

Tested drug instilled as one drop (50μl) twice daily for 7 days in the normotensive left eye and the groups of this part of the study includes :-

- Isotonic Phosphate Buffer group,
- Timolol maleate (0.5%) group
- Nimodipine (0.5%) group

**Effect of the Nimodipine, Timolol and DW on the induced - Ocular Hypertensive Rabbits**

In this part study, ocular hypertension was induced in the left eye, while the right eye left without induction. After proper anesthetization of the left eye by local instillation of 4% lidocaine HCL, a sub conjunctival injection (by using a micro-fine syringes, 30 gauge ×1/2 inches) of 0.7 ml of betamethasone suspension (Celestone Chronodose; Schering-Plough, Madrid, Spain) containing betamethasone sodium phosphate (3 mg/ml) and betamethasone acetate (3 mg/ml). This formulation provides a readily accessible (sodium phosphate) and a sustained release (acetate) fraction of betamethasone. To avoid corneal epithelium damage through too-frequent tonometry, measures of IOP in both eyes were as a rule repeated twice a week, with the first measure being taken immediately before the weekly betamethasone subconjunctival injection and the second considered after 3 days. Three baseline IOP measures were recorded during the week before betamethasone treatment, with animals exhibiting fluctuations of >2 mmHg excluded from the experiments. The value observed at zero time (first betamethasone injection) was considered the starting pressure. All the animals received weekly sub conjunctival doses of betamethasone into the left eye over a period of 4 weeks. The instillation of the tested drugs was started at the 24th day of corticosteroid treatment (3 days after the fourth sub conjunctival injection), a time at which the betamethasone-induced ocular hypertension turned out to be stable, and was prolonged up to 25 days. The experiment model that produced is mimic human chronic open-angle glaucoma (Waleed et al., 2015). The tested drug was instilled as one drop (50μl) twice daily for 7 days (only after the ocular hypertension was definitely established). Each group of this study included six rabbits, and the groups of this part included: DW group, Timolol
maleate (0.5%) group, Nimodipine (0.5%) group.

**Statistical analysis**

The obtained data were presented as mean ± S.E.M. (standard error of the mean). The results were analyzed statistically using Student paired t-test for assessing the effect of the employed drug for a given group of patients. While Student (unpaired) t-test for independent data was used to test the significance of the difference between any two groups. Differences were accepted as insignificant if P > 0.05 and significant P ≤ 0.05 (Daniel, 1983).

**RESULTS AND DISCUSSION**

**Effect of Tested Drugs on Normotensive eyes**

**Effect of Isotonic Phosphate Buffer**

The effect of isotonic phosphate buffer (vehicle) used for the preparation of an ophthalmic solution of the tested drugs on mean IOP of rabbit’s eyes did not reach the level of statistical significance (P ≤ 0.05) during the time course of the experiment (7 days), Figure 1.

**Effect of Distilled Water (Negative Control Group)**

Effect of DW on mean IOP of rabbits eyes nearly remained constant during the time course of the experiment (P > 0.05), Figure 1.

**Effect of Timolol (0.5%) Drop**

The mean IOP value prior commencement of Timolol instillation (0 times or pretreatment value) was (14.2 ± 0.42 mmHg). After 1 hour of single drop instillation, the mean IOP significantly (P = 0.003) decreased to be (12 ± 0.57 mmHg), such reduction was found to be significant (P = 0.02). Compared to that of DW, significant mean IOP reduction (P = 0.03) was found Figure 2. Compared to that of DW a significant mean IOP reduction (P ≤ 0.05) was found on day 1, 2, 3, 4, 5, 6 and 7.

**Effect of Nimodipine (0.5%) Ophthalmic Solution**

The mean IOP value before the commencement of nimodipine instillation (0 times) was (16.5 ± 0.34 mmHg). After 1 hour of single drop instillation, the mean IOP significantly (P = 0.003) decreased to be (14.5 ± 0.43 mmHg) when compared to pretreatment value. Compared to that of DW, there is a significant mean IOP reduction (P = 0.03). Compared to that of Timolol, there was a significant reduction (P = 0.002) in mean IOP. After 1 day of nimodipine instillation (2 times/day), the reduction in mean IOP was (1.5 ± 0.43 mmHg) which was significant (P= 0.017). Maximum mean IOP reduction was (2.7 ± 0.67 mmHg) that achieved in day 4, such reduction was found to be significant (P = 0.01), Figure 3. Along all trial period, mean IOP decreased significantly (P ≤ 0.05) when compared to pretreatment value. Nimodipine could compensate the already detected significant difference (P = 0.006) in pretreatment readings of mean IOP when being compared to that of DW. Such significant difference disappeared on day 1, 2, 3, 4, 5, 6 and 7. Nimodipine could also compensate the already detected significant difference (P = 0.004) in pretreatment readings of mean IOP when being compared to that of Timolol. Such a significant difference disappeared on day 2, 4, and 5.

**Effect of tested drug on experimentally induced ocular hypertension**

The mean IOP of rabbits receiving weekly subconjunctival injections of betamethasone suspension for four weeks gradually increased throughout the experimental period, which became statistically significant since the third day (P= 0.0001) and reached its maximum at the end of the fourth week. This model of induction produced ocu-
lar hypertension mimic human chronic open-angle glaucoma (Mohammed et al., 2017).

**Effect of Distilled Water (Negative Control Group)**

Prior to induction of ocular hypertension, the mean IOP was (15.5 ± 0.47 mmHg). Post induction of ocular hypertension prior to instillation of distilled water, the mean IOP was (21.5 ± 0.33 mmHg); then after DW instillation (twice daily), the mean IOP did not reach the level of statistical significance (P ≥ 0.5) during the time course of the experiment, Figure 4.

**Figure 4: Effect of DW on Mean IOP (mmHg) of Induced-ocular Hypertensive eyes in Rabbits (n=6/group)**

**Effect of Timolol (0.5%) Drop (Response of mean IOP Figure 5)**

At post induction of ocular hypertension, the mean IOP was (23.3 ± 0.84 mmHg). After one hour of single instillation of timolol, mean IOP reduced to be (20.5 ± 0.81 mmHg), such reduction was found to be insignificant (P = 0.09) when compared to that of pretreatment value. Compared to that of DW, there was a significant effect (P = 0.04). Compared to that of Timolol, there was no significant difference (P = 0.5).

After one day of timolol instillation (twice daily), mean IOP significantly (P = 0.027) decreased by (4.5 ± 1.45 mmHg). Maximum decline in mean IOP was (5.3 ± 0.76 mmHg) that achieved in day 4, such decline was found to be significant (P = 0.009). Compared to that of DW group, nimodipine more efficient in days 2 (P = 0.01), 3 (P = 0.02), 4 (P = 0.0005), 5 (P = 0.01) 6 (P = 0.03) and day 7 (P = 0.01), Figure 6. Compared to the Timolol group, no significant difference (P > 0.05) could be detected in mean IOP along all the trial period.

A pilot study was achieved to determine the suitable dose to be tested in the present study, so, the concentration had been selected. The present study clearly demonstrated that a single drop of nimodipine able to reduce mean IOP by (12.12%) in ocular normotensive and by (12.14%) in hyper-
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Figure 6: Degrees of Nimodipine (0.5%)-Induced Changes in Mean IOP (mmHg) of Ocular Hypertensive Rabbits Eyes (n=6/Group).

Other suggested mechanism, nimodipine may interfere with gap junctions (exist between NPE and PE cells), altering cellular permeability of the ciliary epithelium and thus inhibiting normal aqueous humor formation (Green and undefined Kim, 1977).

Elevating of intracellular calcium accelerates the activity of several adenosine triphosphate (ATP) consuming enzymes and one of these enzymes is the enzyme complex Na⁺/K⁺-ATPase (in PE and NPE cells) that transport sodium and potassium ions that are important in aqueous humor formation, thus the use of CCBs play an essential role in impairment of these process (Oram, 2001). In addition to the above mechanisms, the trabecular meshwork cells have contractile properties which may be influenced by calcium ion influx through voltage-dependent L-type calcium channels. Thus the relaxation by CCBs can increase the trabecular outflow facility (Soto et al., 2004). Other probable benefits from application of nimodipine in addition to the ocular hypertensive effect include a potential neuroprotective action against hypoxic retinal ganglion cells and improvement in visual field due to impairment activity of nimodipine to ganglion cell apoptosis which may results from elevation of intracellular calcium ion and the latter stimulates cascade of events resulting in production of free radicals and release of endonucleases, that leading finally to cell death and ganglion cell apoptosis (Dong et al., 2007).
CONCLUSION

Nimodipine has an excellent hypotensive effect on induced-elevated IOP in rabbits.

REFERENCES


