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To evaluate the effect of calcium channel blockers verapamil and diltiazem with timolol on intraocular pressure in rabbits with steroid induced Glucoma: A comparative study

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Abstract

Objective: To evaluate the effect of 0.125% verapamil and 0.5% diltiazem eye drops on intraocular pressure (IOP) in steroid- induced glaucoma in rabbit eyes

Method: A total of 18 rabbits with steroid-induced glaucoma were divided into three groups (A, B and C; n = 6 each). Right eyes in groups A, B and C received 0.5% diltiazem, 0.125% verapamil and 0.5% timolol eye drops twice daily for 12 days, respectively; whereas, left eyes received distilled water. IOP was measured with Tono-pen XL at baseline, day 4, day 8, and day 12 of treatment.

Results: Both 0.5% diltiazem and 0.125% verapamil eye drops significantly reduced IOP compared to control eyes ($p < 0.05$). Reduction of IOP by 0.5% diltiazem, 0.125% verapamil eye drops were comparable to 0.5% timolol. No surface toxicity or systemic side effects were noted during the study period.

Conclusion: Calcium channel blockers, verapamil, and diltiazem significantly reduced IOP in rabbit eyes. This group of drugs may have a potential role in treatment of glaucoma.

Keywords: Calcium channel blockers, Intraocular pressure, Steroid-induced glaucoma.

Introduction

Glaucoma is second leading cause of blindness worldwide.[1] Characterized by progressive degeneration of retinal ganglion cells and optic nerve fibers, leading to

gradual deterioration of visual field. If untreated, it can lead to irreversible blindness.[2]

In most of the cases, glaucoma is associated with high intra- ocular pressure (IOP). Prophylactic medical reduction of IOP reduces the risk of progression to glaucoma from ~10to 5%.[3] There is a constant search for newer drugs that canlower the IOP and therefore possibly retard the progression of glaucomatous optic nerve damage.

Calcium is an important intracellular messenger and Ca^{2+} influx could have several effects on aqueous humor dyna- mics, including hydrostatic component, ciliary perfusion and osmotic component.[4] Calcium channel blockers (CCBs), which are commonly used for the treatment of hypertension and coronary vascular disease, reduce the tone of blood vessels by inhibiting Ca^{2+} influx, causing vasodilation and increasingregional blood flow in several organs including the optic nerve head.[5-10]

Calcium channel blockers may also inhibit the synthesisof extracellular matrix collagen protein, suggesting beneficial effect in glaucoma.[13] CCBs cause relaxation of trabecular meshwork cells by inhibition of L-type channels which increases outflow facility of aqueous humor. The perfusion studies in dissected human eyes showed dose related increase in outflow facility after verapamil administration.[11,12]

In the present study, we investigated the ocular hypotensive role of CCBs in rabbit eyes.

Methods

Study Design: This study was carried out at MGM Medical College, Jamshedpur within a year.

Methodology: The rabbits were inbred in the central animal house under suitable conditions of housing, temperature, ventilation and nutrition. All IOP measurements were obtained with Ton open XL(Reichert Technologies) after anesthetizing the rabbits with 5 mg/ml intravenous midazolam given in dose of 0.5 to 1 mg/kg through marginal ear vein. In addition, topical anesthesia in the form of lignocaine hydrochloride was used before each IOP measurements. An average of three IOP readings was used. Ocular hypertension was induced by bilateral instillation 1% prednisolone acetate eye drops twice a day for a period of 40 days. IOP measurements were obtained before and after treatment with topical corticosteroid eye drops.

Subsequently, the rabbits were divided into three groups and all right eyes in each group received twice daily diltiazem 0.5% eye drops (group A; n = 6) or verapamil 0.125% eye drops (group B; n = 6) or timolol maleate 0.5%eye drops (group C; n = 6) twice daily for 12 days. Sterile distilled water was used twice daily in all left eyes. Diltiazem 0.5% eye drops were prepared by diluting injection diltiazem 25 mg/ml with distilled water upto a concentration of 5 mg/ml. Verapamil 0.125% eye drops were prepared by diluting injection verapamil 2.5 mg/ml with distilled water to a concentration of 1.25 mg/ml.

IOP was measured in both eyes before instilling these drugs and on every 4th day till the end of 12 days of treatmentperiod.

Sample Size: A total of 18 albino rabbits (aged 3-4 months) of either sex weighing 1.5 to 2.5 kg were used in this study.

Statistical analysis: Results were expressed as mean ± SD and percentage changes wherever required. Intragroup comparisons were performed using the t-test. One-way analysis of variance was used for multiple group comparisons followed by posthoc Tukey's test for group-wise comparisons. A 'p-value of 0.05 or less was considered for statistical significance.

Results

Mean basal IOP increased in all three groups after 40 daysof twice daily treatment with 1% prednisolone acetate eye drops (Tables 1 to 3).

Table 1: Mean basal, post-topical corticosteroid and post-topical diltiazem treatment intraocular pressure in group A rabbits

	Baseline	After corticosteroid	Day 4	Day 8	Day 12
OD diltiazem	16.4 ± 1.4	25.5 ± 1.6	22.9 ± 1.7	17.9 ± 1.3	16.9 ± 1.1
OS control	18.0 ± 2.3	25.9 ± 1.9	25.9 ± 1.9	25.2 ± 1.7	24.8 ± 1.7

Table 2: Mean basal, post-topical corticosteroid and post-topical verapamil treatment intraocular pressure in group B rabbits

	Baseline	After corticosteroid	Day 4	Day 8	Day 12
OD verapamil	16.4 ± 1.4	24.5 ± 1.0	22.7 ± 1.3	18.0 ± 2.3	15.5 ± 1.4
OS control	15.5 ± 1.4	25.9 ± 1.9	25.9 ± 1.9	25.5 ± 1.6	25.5 ± 1.6

Table 3: Mean basal, post-topical corticosteroid and post-topical timolol treatment intraocular pressure in group C rabbits

	Baseline	After corticosteroid	Day 4	Day 8	Day 12
OD timolol	16.0 ± 1.5	22.4 ± 1.9	22.4 ± 1.9	19.0 ± 1.8	16.4 ± 1.4
OS control	16.4 ± 1.4	26.2 ± 2.1	26.2 ± 2.1	25.5 ± 1.6	25.5 ± 1.6

All groups were comparable in terms of pre- and post corticosteroid treatment ($p > 0.05$). Group A (diltiazem 0.5%) eyes did not show any statistically significant reduction in the IOP in the left eyes (controls) up to day 12. However, the IOP reduced in the right eyes (treatment) starting from day 4.

There was astatistically significant difference in the mean IOP treatmentand control eyes in group A ($p = 0.0153$). The control eyesin group B did not show a significant reduction in the IOP over the study period. The treatment eyes showed a significant reduction in the mean IOP level on days 4, 8 and 12 ($p = 0.0171$). Similar results were obtained in the treatment and control eyes of group C ($p = 0.0192$). Further, there was no statically significant difference in the IOP lowering effect of all three drugs (post hoc Tukey's test).During the study period, no ocular surface toxicity or systemic side effects were noted in any of the rabbits.

Discussion

Most of the previous studies have employed normal/low tension glaucoma animal models to demonstrate the effects of topical CCBs on IOP. In the present study, we demonstrated a reduction in corticosteroid induced ocular hypertension with topical calcium channel blocking drugs. The hypotensive effect was comparable to that of topical timolol eye drops.[13]

Calcium channel blockers alter the intracellular calcium concentration by modifying calcium flux across cell mem- branes and affect various intracellular signaling processes.[14,15] Lipid soluble CCBs act at the central nervous system level, whereas water soluble CCBs act mainly on the cornea and opticnerve.[16] It is also known that calcium influx is the terminal step in axonal death in the glutamate pathway. The ability toblock calcium influx can, therefore, produce a neuroprotectivebenefit.[17] Furthermore, CCBs can improve ocular blood flowthrough inhibition of

endothelin-1.[18-21] Despite this, the effect of CCBs on IOP remains controversial.[22-27]

Calcium influx could have several effects on aqueous humor dynamics, including a hydrostatic component caused by an effect on arterial blood pressure and ciliary body perfusion, and an osmotic component caused by an effect on theactive secretion of sodium, calcium and other ions by ciliary epithelium.[28] Recent reports have addressed the effect of CCBs on ocular blood flow. Using laser Doppler velocimetryand flowmetry in cats, Harino et al demonstrated increased optic nerve head blood flow following administration of intravenous nicardipine.[29] Net land et al utilized color Doppler ultrasound analysis and found that topical verapamil maydecrease the vascular resistance in ocular blood vessels.[26]

Favorable effects of CCBs on visual field defects as wellas contrast sensitivity have also been reported.[29-31] Verapamil tends to block both activated and inactivated L-type calcium channels. It has also been shown to improve the blood supply in rabbit eyes with experimental glaucoma acting as vasodilator and improving the outflow facility.[32] Diltiazem, on the contrary, has been shown to produce relaxation of serotonin- induced contraction of bovine ophthalmic artery primarily by inhibiting the Ca^{2+} influx.[33] It was shown to exhibit a long lasting and dose related effect on IOP.[34] CCBs may, therefore, play a potential role in relaxing the retinal, long posterior ciliary, and ophthalmociliary arteries to improve the ocular circulation in vascular diseases in which considerable vascular tone is present.[35] Santafe et al reported that CCBs decrease aqueous humor secretion in addition to causing a slight but significant reduction in tomographic outflow facility.[34] Also, the outflow of aqueous humor influenced by episcleral venous pressure may be directly affected by calcium inhibition. Verapamil may interfere with gap junctions between nonpigmented and pigmented ciliary epithelial cells altering cellular permeability of the ciliary epithelium and thus inhibiting normal aqueous humor formation.[34,36] It may also alter the cyclic adenosine monophosphate content in ciliary epithelial cells, thereby affecting IOP through a decrease in aqueous humor formation, or an increase in outflow facility.[37]

Lowering of lOP by verapamil and diltiazem may be due to inhibition of the intracellular uptake of calcium by inactivating the inner phosphorylation dependent calcium gate of the cellular membrane.[10] It is known that trabecular meshwork cells have contractile properties, which may be influenced by Ca2+ influx through voltage dependent L-type Ca^{2+} channels. These agents cause relaxation of trabecular meshwork cells and increase the outflow facility. The perfusion studies in dissected human eyes showed dose related increase in outflow facility after verapamil administration.[38]

Calcium channel blockers cause vasodilatation and reduce vascular resistance, increase the capillary bloodspeed in the optic nerve head, this make them to be possible drugs useful in the treatment of low-tension glaucoma.[10] The results of our study match the earlier reports that showed that topical application of verapamil and diltiazem effectively lowered IOP in a dose related fashion.[24,34]

Topical verapamil has also been shown to reduce IOP in humans.[7,26,39] A single topical application of 0.125% verapamil prompted a 3 to 4 mm Hg IOP decrease in 12 ocular hypertensive patients that lasted up to 10 hours,[7] whereas a slight reduction (≈1.5 mm Hg) was noted in normal volunteers.[26] After topical application of 0.125% verapamil for 2 weeks, a 7.0 ± 2.9 mm Hg

decrease in IOP has been measured in ocular hypertensive subjects.[8]

Conclusion

Our study highlights the potential role of CCBs in management of corticosteroid induced glaucoma in rabbit eyes. CCBs were comparable with commonly used beta blocker drug. Nevertheless, further studies are needed to replicate the ocular effects of CCBs in humans and determine their potential clinical use in glaucoma patients.

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