Original Article

Topical Diltiazem Vs Travoprost in Reducing Intraocular Pressure in Ocular Hypertensive / Glaucomatous Rabbits

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Purpose: To demonstrate the Intraocular Pressure (IOP) lowering effect of topical Diltiazem (calcium channel blocker) in comparison to Travoprost (anti-glaucoma drug).

Material and Methods: The study was conducted on 50 healthy rabbits of local strain, weighing 1500 to 2000 grams. They were kept at the animal house of Department of Pharmacology, Khyber Medical College Peshawar. Effect of drugs was studied on both eyes of conscious rabbits. Rabbits were divided into four groups i.e. A, B, C and D. Rabbits of group A, B and C were made ocular hypertensive / glaucomatous by injecting weekly sub conjunctival betamethasone suspension. The iatrogenic glaucoma of group "A" animals were treated with topical Diltiazem and group B with topical Travoprost drops. Group C served as ocular hypertensive control. It received only artificial tears during the research period. Group D rabbits were used as normotensive control. They were neither induced for glaucoma nor did they receive any treatment during the research period.

Results: Our study revealed that there was 19% (5.00 $\pm$ 0.25 mm Hg) drop in Intra Ocular Pressure with topical Diltiazem. Its onset of action was quick and duration of action prolonged. Whereas topical Travoprost reduced IOP by 15% (4.00 $\pm$ 0.25 mm Hg). Topical Diltiazem was found consistent in its intraocular pressure lowering effect as compared to Travoprost.

Conclusion: Topical Diltiazem can be used as an alternative anti glaucoma drug in future if found safe in human trials.

Key words: Glaucoma, Ocular hypertension, Betamethasone suspension, Calcium Channel Blockers (CCB), Intraocular Pressure (IOP).

Human nature is curious and non-satisfying. A global research is always underway to find new and improved treatment of glaucoma. As per Glaucoma continuum, clinical picture of glaucoma is quite horrible and unpredictable. Extensive multicenter researches are in the pipe line to find out the exact mechanism of glaucoma and also to improve anti glaucoma therapy.[9]

Allingham and M Bruce Shield have mentioned various groups of drugs that are under investigation having not only intraocular ocular pressure lowering properties but also vasodilating and neuroprotective effects.[2]

Calcium channel blockers (CCB) are diverse group of drugs,[16] whose therapeutic utilities are still to be explored to fully unleash its therapeutic effectiveness. The new millennium will hopefully explore their diversity in various medical specialties including ophthalmology and especially in glaucoma.

Since 1970's CCBs are being tested for their effects on IOP. An ample literature is available on the

intraocular pressure affecting properties of CCB's. There are several conflicting reports available regarding the effects of CCB's on IOP,[18,20,23] but the general tendency is towards its intraocular pressure lowering effect.[1,21,22]

In glaucoma, the exact mechanism of calcium channel blockers (CCB's) on intraocular pressure regulation is not known however literature search has revealed following possible potential mechanisms:

1. CCB's act on vascular smooth muscles causing vasodilatation thereby improving optic nerve blood flow and on intracellular calcium metabolism causing neuroprotection.[19]
2. CCBs cause reduction in aqueous humor production by affecting the ultra-filtration of aqueous in ciliary processes. This is done by relaxing blood vessels in ciliary epithelium thus decreasing the hydrostatic pressure which is one of the factors that causes passage of fluid into ciliary processes.[10]
3. The (Gap) junctions which are possibly regulated by calcium, exist between non pigmented and pigmented ciliary epithelial cells, CCBs may interfere with these (Gap) junctions, altering cellular permeability of the ciliary epithelium and thus inhibiting normal aqueous humor formation.[11]
4. The potassium channel is important in formation of aqueous humor in ciliary epithelium, and this channel depends on the calcium ion. Topical administration of the calcium ions has shown an increase in the IOP.[12] For the reason the CCBs tend to cause reduction in aqueous formation.
5. The trabecular meshwork cells have contractile properties which may be influenced by calcium ions influx through voltage-dependent L-type calcium channels, thus the relaxation of meshwork by CCBs can increase the trabecular outflow facility.[13] The perfusion studies in dissected human eyes showed dose related increase in outflow facility after Verapamil, Diltiazem and nifedipine administration. However in addition, the outflow of aqueous humor influenced by episcleral venous pressure may be directly affected by calcium inhibition.[14]

In 1997 steroids in suspension form were used to raise intra ocular pressure.[15]

The present study has been designed to see the effectiveness of topically applied Diltiazem on steroid induced raised intraocular pressure in an animal model. The result of the study will be an addition to the existing data and will help in the development of new drug for glaucoma therapy for human beings.

Ethical approval for this study was obtained from the college ethical committee.

MATERIAL AND METHODS

The study was done on both eyes of conscious and normal 50 rabbits. Rabbits of either sex i.e. male / female and of both species i.e. colored and albino were used. Ages of rabbits were between 1 – 2 years and weight in the range of 1500 – 2000 grams. They were observed for 02 weeks before experimentation. Rabbits were kept in the animal house of Department of Pharmacology, Khyber Medical College Peshawar. Fresh and wholesome was provided ad libitum. Animals were also provided fodder, wheat grains and grams ad libitum.

Rabbits were divided into four groups A, B, C and D.

GROUP 'A' consisted of 10 steroid induced ocular hypertensive rabbits. These animals were treated with topical Diltiazem 8.9×10^{-2} M, 1 drop daily for 04 weeks.

GROUP 'B' consisted of 10 ocular hypertensive rabbits treated with topical Travoprost 1 drop daily for 04 weeks.

GROUP 'C' consisted of 20 ocular hypertensive rabbits that served as ocular hypertensive control. This group received artificial tears 1 drop daily for 04 weeks.

GROUP 'D' consisted of 10 rabbits, used as normal control i.e. normotensive. It received no treatment during the entire period of study.

The study was conducted in the Department of Pharmacology, Khyber Medical College Peshawar in two phases i.e. Phase - I and Phase - II.

PHASE-I (Ocular hypertensive phase):

During this phase, rabbits of group A, B and C were made ocular hypertensive. Rabbits of group D served as normal control. This phase lasted for 21 days i.e. 03 weeks (day 0 to day 21).

PHASE-II (Treatment Phase):

There was a gap of 02 days (day 22 and day 23) to get a fully established raised intraocular pressure, prior to the start of phase-II.

During phase II, animals of group A, B and C received topical treatment.

Animals of group 'A' were instilled topical Diltiazem 8.9 x 10^{-2}M solution, group 'B', Travoprost drops and group 'C' artificial tears in their eyes.

All the drugs were instilled in the frequency of 1 drop daily for 28 days i.e. 04 weeks (day 24 to day 51).

REAGENTS AND DRUGS

1. Diltiazem powder (I Golani Traders, Chandi Ghar, India).
2. Travoprost solution; 0.004% (Travatan; Alcon-Couvereur, Belgium).
3. Proparacaine HCl 0.5% (Alcaine; Alcon – Couvereur, Belgium).
4. Injection Betamethasone suspension (Celestone Cronodose; Schering – Plough, Spain).
5. Artificial tears drops (Alcon – Couvereur, Belgium).
6. Fluorescein Sodium 2% (Alcon – Couvereur, Belgium).

Diltiazem is available only in tablet form in different strengths as Diltiazem HCL. This drug is not available as ophthalmic preparation for therapeutic or experimental purposes.

A solution of 8.9 x M strength was chosen. It is the strength which has been reported to induce IOP lowering effectively[15]. Its molecular weight is 450.98.

4.013 grams of Diltiazem powder was dissolved in 100 ml of distilled water. It served as the stock solution. It was refrigerated and used during the study as a drug per instillation schedule

INDUCTION OF OCULAR HYPERTENSION/ GLAUCOMA

1. Group 'A', 'B' and 'C' animals (n = 40) were made ocular hypertensive.
2. IOP was raised by subconjunctival injection of steroids in the suspension form.[24]

   To administer injection rabbits were held in especially designed wooden boxes. Both the eyes of rabbits were anesthetized by instilling 1 drop of 5% proparacaine HCl every 15 seconds for one minutes. After two minutes betamethasone suspension was injected into the subconjunctival sac using insulin syringe. Mild pressure was applied on the eyes for a short period to enhance absorption of drug.
3. In our study rabbits were given weekly subconjunctival injection of 0.7 ml solution of betamethasone sodium phosphate and betamethasone acetate 3 mg/ml each in both eyes for 3 weeks. Total of three injections were administered.
4. This combination provided a slow released acetate fraction of betamethasone and readily available sodium phosphate.

MEASURMENT OF INTRA OCULAR PRESSURE

Before starting the study, IOP of all rabbits were measured for 2 weeks. 04 measurements were taken during this time. Animals exhibiting fluctuations > 5 mm Hg in their IOP were excluded from the study (n = 5). The excluded animals were replaced with new set of rabbits.

Intraocular pressure was measured with Perkins hand held applanation tonometer (Clement Clark Int. Ltd. Essex England).

Throughout the study IOP was measured twice a week only i.e. on Thursday and Monday to avoid corneal epithelial damage and at the same time i.e. 9:00 AM, to avoid diurnal variation.

During Phase-I, the 1st reading of IOP was taken immediately before injecting weekly Betamethasone i.e. Thursday and 2nd was recorded after 3 days i.e. Monday.

The values observed at "zero time" i.e. 1st injection of Betamethasone were considered the base line pressure.

Animals were placed in especially designed containers to reduce movements. Eyes of rabbits were anesthetized with topical local anesthesia and cornea stained with fluorescein.

During Phase-II, steroid was stopped but measurement of IOP continued. IOP values observed at the start of phase-II were considered to be the starting pressure.

Instillation of Diltiazem, Travoprost and artificial tears were started during phase-II at the 24th day of study (02 days after 3rd betamethasone suspension injection).

IOP was recorded before instillation of drugs on Monday and Thursday at 9.00 AM.

RESULTS

The IOP measurements of 50 rabbits were recorded as shown in table 1 and 2.

Table 1: Mean IOP's of group A and B in comparison to group C (the ocular hypertensive control).

Week	Topical Treatment			P-value (ANOVA Test)
	Artificial Tears	Diltiazem	Travoprost	
	Group C (n = 20)	Group A (n = 10)	Group B (n = 10)	
0	26.51 ± 0.22	26.37 ± 0.24	26.42 ± 0.23	0.25959
1	26.52 ± 0.30	25.45 ± 0.25	26.00 ± 0.23	0.0000000000147759
2	26.51 ± 0.24	21.27 ± 0.69	24.05 ± 0.36	0.0000000000
3	26.35 ± 0.39	21.00 ± 0.77	22.22 ± 0.41	0.00000
4	25.35 ± 0.31	20.50 ± 0.66	20.70 ± 0.43	0.00000

Table 2: Comparison of mean IOP differences between group A and B.

Week	IOP		P-value (ANOVA Test)
	Diltiazem	Travoprost	
	Group A	Group B	
0	26.37 ± 0.30	26.42 ± 0.25	0.6903
1	25.45 ± 0.25	26.00 ± 0.23	0.00003435
2	21.27 ± 0.69	24.05 ± 0.36	0.0000001
3	21.00 ± 0.77	22.22 ± 0.41	0.0003287
4	20.50 - 0.66	20.70 ± 0.43	0.4325

The overall normal IOP (n = 50) before the start of injecting steroid was in the range of 19.50 ± 0.75 to 21.75 ± 0.25. Mean pre-steroidal baseline pressure was 20.83 ± 0.75. Injection of steroids led to a rapid rise in IOP of group A, B and C. The rise in IOP was found statistically significant after 2nd injection of betamethasone suspension and highly statistically significant after 3rd injection. The normotensive control (group D), did not show any statistically significant change in their IOP throughout study ($P > 0.05$). Their pressure was in the range of 20.62 ± 0.65 to 21.07 ± 0.37.

Table 1 represents mean IOP ± SD of group A, B and C rabbits. Topical Diltiazem and Travoprost reduced IOP effectively. The change in IOP of group A and B in comparison to group C, became highly statistically significant right from the 1st week of treatment and remained so throughout the observational period ($P < 0.00$).

With reference to Table 2, topical Diltiazem proved to be efficacious in its IOP lowering effect. It dropped the IOP very briskly, particularly between week 1 and 2. Amazingly, this IOP lowering was so efficacious that, during week 4, it even dropped (20.50 ± 0.66) below base line IOP's lowest observation of 20.62 ± 0.65 ($P < 0.05$). The IOP, between week 3 and 4, was maintained at a constant level. Interestingly IOP drop became statistically non-significant in the last week of treatment ($P > 0.05$).

Travoprost efficaciously dropped IOP ($P < 0.05$). Onset of action was rather slower but gradual as compared to topical Diltiazem. It was found consistent in its IOP lowering effect during entire study with an average 1.90 ± 0.45 mm Hg drops per week $P < 0.00$.

IOP lowering reached base line value during 4th week of treatment (Table 1 and 2). This finding is not in parallel with Diltiazem, in which, IOP touched base line during 2nd week.

Topical Diltiazem demonstrated an acute IOP lowering potential, 5.10 ± 0.61, between week 0 and 2; with an average 1.38 ± 0.45 mm Hg drop during week 3 and 4. It seemed that Diltiazem took about 02 weeks to fully establish its IOP lowering effect.

Topical Diltiazem became statistically insignificant ($P > 0.066$) between week 2 and 3 and resumed its IOP decremental activity between week 3 and 4. The duration of action of both drugs was found prolonged i.e. 24 hours and with an early onset of action.

DISCUSSION

CCB's are being investigated for more than three decades for their IOP lowering effects. An ample data is available regarding IOP lowering potential of calcium channel blockers. The ocular effects of CCB's have been reported since 1970's. It has been reported in humans, ocular normotensive and ocular hypertensive animals. Results are conflicting and till date no consensus has been made.[5,18,20,23]

Above all, CCB's are still in the main stray of researchers because of their greater positive potential to affect glaucoma patients by not only lowering IOP but also providing vasodilatation and neuroprotection.[3,4,7,17] American Glaucoma Society in its 22nd annual meeting has linked use of calcium and iron supplementation in glaucoma patients.[6]

This study revealed that Diltiazem can lower IOP effectively and briskly thus, leading to an addition in the existing data that favors CCB's role in the management of glaucoma / ocular hypertension.

Melena, et al, described the ocular hypotensive effect of CCBs in rabbit model for glaucoma.[25] A single dose of verapamil, nifedipine and Diltiazem produced a dose – dependent decrease in IOP in ocular normotensive rabbits after topical application but not after intravenous administration.[25] Furthermore, the ocular hypotensive effect of Diltiazem was remarkable due to its duration, thus permitting the appropriate administration frequency.[26]

In humans, topical verapamil, Diltiazem and nifedipine have been found to significantly lower IOP in normal and ocular hypertensive subjects.[27]

A single topical application of CCBs prompted IOP decrease in ocular hypertensive patients. It was also found that verapamil and Diltiazem significantly lowers the IOP in normal human volunteers.[28]

CONCLUSION

Diltiazem may be helpful in treatment of acute ocular hypertensive crises due to its brisk IOP lowering effects and in the treatment of glaucoma and ocular hypertension.

However further laboratory and animal studies are required to explore the exact IOP lowering mechanism of action, vasodilation and neuroprotection properties of topical Diltiazem and demonstrate its systemic or local untoward effect.

Detailed controlled clinical studies using Diltiazem (0.25%) or (0.5%) eye drops for patients with glaucoma seemed to be recommended.

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