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THE EFFCETIVENESS OF TOPICAL DILTIAZEM IN REDUCING INTRAOCULAR PRESSURE

Saadat Ullah Khan, Mohammad Abid Shah, Sartaj Khan, Ibrar Hussain

Department of Pharmacology, Khyber Medical College, Peshawar - Pakistan

ABSTRACT

Objective: To see Intraocular Pressure (IOP) lowering potential of topical diltiazem, a calcium channel blocker in glaucomatous rabbits.

Matereial and Methods: Forty healthy rabbits of a local strain weighing 1.50 to 2.00 kilograms were obtained and kept at the animal house of the Department of Pharmacology, Khyber Medical College, Peshawar. The study was conducted from April 2013 to May 2013 on both eyes of conscious rabbits. Three groups of animals were constituted i.e. Group A, B and C. Group A animals, made ocular hypertensive/ glaucomatous by injecting weekly subconjunctival betamethasone suspension, were administered topical diltiazem. Group B was also made ocular hypertensive and served as the ocular hypertensive control. It received artificial tears for 4 weeks during the entire research period. And group C was used as the normotensive control. It received no treatment during research.

Results: It was found that topical diltiazem led to the reduction in the intraocular pressure very effectively and briskly. Topical diltiazem exerted intraocular pressure lowering effect in a very brief period of time. It was also associated with tremendous animal survivability.

Conclusion: Topical diltiazem can be used as an alternative anti glaucoma particularly to address ocular hypertensive crises drug in future if found safe in human trials.

Key Words: Betamethasone, Glaucoma, Ocular hypertension, Calcium channel blockers, intra occular pressure.

INTRODUCTION

Human nature is non satisfying and curious. A global research, in the same context, is underway to improve the treatment of glaucoma. Extensive and multicenter researches are in the pipe line to discover the exact cause and mechanism of development of glaucoma and also to improve anti glaucoma therapy.[1] Allingham and M Bruce Shield has mentioned many groups of drugs that are being thoroughly investigated that will not only have intraocular ocular pressure lowering property but will also have vasodilating and neuroprotective effects.[2] As per Glaucoma Continum, clinical picture of glaucoma is quite horrible and unpredictable.

Calcium channel blockers are diverse group of drugs[3]. With the lieu of therapeutic utilities of CCB's more and more avenues are still to be explored to fully unleash therapeutic effectiveness of CCB's. The new millennium will hopefully explore their diversity in many medical specialties including ophthalmology.

Since 1970's CCBs are being tested for their effects on IOP. An ample literature is available about IOP affecting property of CCB's. There are several conflicting reports available regarding the effect of CCB's on IOP[4,5,6] but the general tendency is towards a decrease in IOP[7,8,9].

In glaucoma, calcium channel blockers have three potential applications for their effect on IOP: on vascular smooth muscles i.e. vasodilatation and thereby, improving optic nerve blood flow and on intracellular calcium metabolism i.e. neuroprotection (Ocular Pharmacology 1997). The procedure to raise IOP was by using steroids in suspension form was as described by Santafe J et al[10]. 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 lead to an addition in the existing conflicting data.

Address for Correspondence:
Dr. Saadat Ullah Khan
Assistant Professor
Department of Pharmacology,
Khyber Medical College, Peshawar - Pakistan
Contact: 0333-9138116
Email: saadat2k1@yahoo.com

MATERIAL AND METHODS

The study was conducted on rabbits in two steps i.e. phase-I and phase-II from April 2013 to May 2013. During this phase the rabbits of group A and B were made ocular hypertensive, except the normotensive control group C. The phase was consisted of 03 weeks i.e. 21 days (0-21). Prior to the start of phase-II, there was a gap of 02 days, to get a fully established raised intraocular pressure (day 22 & 23). During phase-II, rabbits of group A, made ocular hypertensive during phase-I, were given treatment with topical diltiazem 8.9 x 10^{-2}M solution. Group B was instilled artificial tears. The phase was consisted of 04 weeks i.e. 28 days (day 24-51). Both the drugs were instilled 01 drop 07 days

a week. The study sample consisted of 40 rabbits. It was conducted on both eyes of normal and conscious rabbits. Rabbits of either sex i.e. male and female and of both species i.e. albino and coloured strains were used. Weight of rabbits was between 1.50–2.00 Kgs and age was between 1-2 years. They were observed for 2 week before experimentation. They were kept in the animal house of Khyber Medical College Peshawar. The animals were fed on fodder, wheat grains and grams, ad libitum. Fresh and wholesome water was also provided ad libitum.

Three groups of rabbits were constituted. Group A: This group was consisted of 10 steroid treated ocular hypertensive rabbits. It was instilled topical diltiazem 8.9 x 10^{-2} M for 04 weeks. Group B: Group B was consisted of 20 rabbits. It was also made ocular hypertensive. It served as the ocular hypertensive control. It received artificial tears for 04 weeks. Group C: It was consisted of 10 rabbits. This group was used as normal control i.e. normotensive. It received no treatment during the entire period of study. The following chemicals were used: Diltiazem powder (I I Golani Traders, Chandi Ghar, India), Proparacaine HCl 0.5% (Alcaine; Alcon – Couvereur, Belgium), Inj Betamethasone suspension (Celestone Cronodose; Schering – Plough, Spain), Fluorescein Sodium 2% (Alcon – Couvereur, Belgium), Artificial tears drops (Alcon – Couvereur, Belgium). The following equipments were used: Perkins hand held applanation tonometer (Clement Clark Int Ltd. Essex England), Rabbits boxes/ containers. The rabbits were held in especially designed wooden boxes. It was instilled 1-2 drops 5% proparacaine HCl to induce local anesthesia. After a few minutes betamethasone suspension was injected in subconjunctival sac of the rabbits. Insulin syringes were used to inject the drug. Mild pressure was applied on the eyes for a short period of time, Before starting the study, the IOP's of all rabbits were taken with tonometer for 02 weeks. 04 measurements of the IOP were taken during this time. Animals exhibiting fluctuations >5mm Hg in their IOP were excluded from the study (n = 5). New set of animals was included to replace the excluded ones, To avoid diurnal variation of the IOP, measurement was almost always, started at the same time of the day (9:00 AM) throughout the observational period (Text Book of Ocular Pharmacology 1997), Measurement of the IOP in both eyes was performed, as a rule, twice a week. This helped to avoid corneal epithelial damage (Kanski 2004). It was done on Thursday and Monday, During phase-I, 1st measure 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, Before taking the measurements, the rabbits were given topical local anesthesia followed by fluorenscein causing staining of cornea, Then, the animals were placed in especially designed containers.

Table 1: Mean IOP's of group B and group C during phase-I and II

Time Interval (weeks)	Group B (Ocular hypertensive)	Group C (Normotensive control)
0	20.83±0.75	21.00 ±0.20
1	21.03±0.75*	20.62± 0.65
2	21.75±0.30**	20.70±0.30
3	23.01±0.60**	20.87±0.45
4	26.51±0.22**	20.92±0.58
5	26.52±0.30**	20.90±0.61
6	26.51±0.24**	21.00±0.50
7	26.35±0.39**	21.05±0.48
8	25.35±0.31**	21.07±0.37

Table 2: Mean IOP differences of group A and B during phase-II

Time Interval (Weeks)	Group A (Topical diltiazem)	Group B (Artificial tears)
0	26.37± 0.24	26.51± 0.22
1	25.45 ±0.25**	26.52 ±0.30
2	21.27 ±0.69**	26.51± 0.24
3	21.00 ±0.77**	26.35± 0.39
4	20.50 ±0.66**	25.35 ±0.31

Table 3: Week wise mean IOP difference of diltiazem treated ocular hypertensive rabbits

	Time Interval (Weeks)	Group A (Diltiazem treated)	Mean difference
0	Starting IOP	26.37±0.30	0.92 ± 0.21
	Week 1 / Reading 2	25.45±0.25	
1	Week 1 / Reading 2	25.45±0.25	3.18 ± 0.61**
	Week 2 / Reading 2	21.27±0.69	
2	Week 2 / Reading 2	21.27±0.69	0.27 ± 0.41 **NS**
	Week 3 / Reading 2	21.00±0.77	
3	Week 3 / Reading 2	21.00±0.77	0.50 ± 0.65*
	Week 4 / Reading 2	20.50±0.66	

NB: 2nd measure of IOP has been mentioned only

This caused the animals to remain unmoved, Then, by applying applanation tonometer the IOP of the rabbits was recorded, During phase-II, steroid was stopped. But, measurement of the IOP was continued. IOP was recorded now, before the instillation of drugs on Thursday and Monday at 9.00 AM, IOP values observed at the start of phase-II were considered to be the starting pressure.

Diltiazem is available only in tablet dosage form in different strengths as Diltiazem HCl. There is no availability of ophthalmic preparation for therapeutic or experimental purposes. A solution of 8.9×10^{-2}M was chosen. It is the strength which has been reported to induce IOP lowering effectively[11]. Its molecular weight is 450.98[12] 4.013 grams of diltiazem powder was dissolved in 100mls of distilled water. It served as the stock solution. It was refrigerated and used during the study as drug instillation schedule.

RESULTS

The IOP measurements of 40 rabbits were recorded. Data of both eyes was found similar and here, the data of right eye has been mentioned only. The measurements were found to be as shown below with reference to *, ** and NS, * Significant ($P<0.05$),** Highly significant ($P<0.05$), NS Non significant ($P>0.05$). Table 1 shows mean intra occular pressures of group B and C during phase 1 and 2, Table shows mean intra occular pressures differences of group A and B during phase 2. Table 3 shows weak wise mean intra occular pressure difference of Diltiazem treated occular hypertenstive rabbits.

DISCUSSION

CCB's are being investigated for more than 03 decades for their IOP lowering effect. An ample data is available regarding IOP lowering potential of calcium channel blockers. The ocular effects of CCB's have been reporting 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[13,14,15].

Above all, even then, CCB's are still in the main stray of the researcher because of their greater positive potential to affect glaucoma patients not only to lower IOP but also providing vasodilatation and neuroprotection[16,17,18,19].

The purpose of present study was to demonstrate IOP lowering property of topical diltiazem, if any. This study proves that diltiazem can lower IOP, thus, leading to an addition in the existing data that favours CCB's role in management of glaucoma/ ocular hypertension.

The overall normal IOP ($n=40$) before the start of steroid treatment was in the range of 19.50 ± 0.75 to 21.75 ± 0.25. Mean pre-steroidal baseline pressure was 20.83 ± 0.75. Injectable steroid led to a rapid rise in IOP of group A and B. The gain in IOP was found statistically significant after 2nd dose of betamethasone suspension with a P value <0.05. The elevation became highly statistically significant after 4th injection ($P<0.00$).

The normotensive control, group C, did not show any statistically significant change in their IOP'S throughout study ($P>0.05$). Their pressure was in the range of 20.62 ± 0.65 to 21.07 ± 0.37.

When the results obtained after the 2nd phase of study are interpreted it becomes evident that topically applied diltiazem reduced the IOP effectively (Table 2). Group A result shows highly statistically significant values ($P<0.00$) as compared to the ocular hypertensive control group B. The starting pressure was 26.37 ± 0.24, while the final reading was 20.50 ± 0.66.

The change in IOP of group A in comparison to group B became highly statistically significant right from the 1st week of treatment and it remained ($P<0.00$). Topical diltiazem proved to be efficacious in its intraocular pressure lowering action. Topical diltiazem drop the IOP very briskly, particularly between week 1 and 2. The IOP, between week 3 and 4, was maintained at a constant level. An acute drop in the IOP noticed was 5.10 ± 0.61 between week 0 and 2. And 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 ($P<0.05$). It became statistically non significant in the last week of treatment ($P>0.05$), when its IOP lowering was compared week wise (Table 3).

Diltiazem was applied once daily which effectively controlled the IOP. The duration of action of topical diltiazem was found prolonged i.e. at least 24 hours and with an early onset of action. The drop in IOP was found statistically, consistently, highly significant ($P<0.00$) throughout the observational period (Table 2 and 3).

After discontinuation of injection betamethasone some spontaneous IOP lowering was also noticed in group B. The drop in IOP was found statistically significant ($P>0.05$) as compared to the values observed at the end of steroid therapy (Week 3). After cessation of betamethasone therapy, the IOP was monitored for further 04 weeks in both groups. IOP lowering was observed during this period (Data not mentioned).

CONCLUSION

Diltiazem may be helpful in treatment of acute ocular hypertensive crisis due to its brisk IOP lowering effects and as well, in the treatment of glaucoma and ocular hypertension. Dose adjustment must be mandatory to manage these illnesses.

Diltiazem, in its solution dosage form, also needs to be tested in human volunteers and then in glaucoma/ ocular hypertension patients. Topical diltiazem effect on vasodilatation and nerve protection obviously needs further high profile studies.

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