Original Article

IOP Lowering with Beta Blocker

Novel Role of Topical Diltiazem in Reducing Raised Intraocular Pressure in Rabbits

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ABSTRACT

Objective: The objective of the current study was to evaluate the IOP lowering of topical diltiazim which is a calcium channel blocker.

Study Design: Observational / descriptive study

Place and Duration of Study: The study was conducted at the Department of Pharmacology, Khyber medical college Peshawar, KPK-Pakistan from November 2015 to February 2016.

Materials and Methods: 40 healthy rabbits of a local strain weighing 1.50 to 2.00 kgs were obtained and kept at the animal house of the department of pharmacology, BMC Bannu. The study was conducted on both eyes of conscious rabbits. Three sets namely X, Y&Z were made. Topical diltiazim was injected to set X(made ocular hypertensive and glaucomatous through weekly injecting sub-conjunctival betamethasone suspension). Ocular hypertensive control set Y was also established which gotsynthetic tears for a period of 28 days through the whole project. SetZ received no treatment during research and it act as normotensive control.

Results: Our results indicated that topical diltiazim can reduce the intraocular pressure very efficiently and quickly. Topical diltiazim wielded IOP reducing outcome in a much transitory time retro. Marvelous animal's survival was also related to it.

Conclusion: In future, topical diltiazim might be incorporated as asubstitute anti-glaucoma drug in order to manageoptical hypertensive crisis, provided its safety in human.

Key Words: Glaucoma, Optical Hypertension, CCB, IOP

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INTRODUCTION

Man is borne with a nature which is non-satisfying and inquiring. That is why he is always been involved in newer researches. A worldwide research in the same background, is ongoing to expand the management of glaucoma. To make improvement in the treatment of glaucoma and to explore the causes being involved in its onset, researchers are making extensive work on the same[1]. A no of drugs are made which have vasodilating and intraocular pressure lowering tendencies[2]. As per Glaucoma Range, medical scenario of the disease is fairlyterrible and capricious.

CCB or Calcium channel blockers are assorted collection of drugs[3]. Keeping in view the therapeutic values of CCB's, many boulevards are still need to be discovered in order to completely understand salutary effectiveness of CCB's. In coming years, research will expectantly discover their variety in numerous therapeuticarenas additionally with ophthalmology.

CCBs are in use since 70's for their role in lowering IOP. An abundant research articles are accessible about IOP upsetting possessions of CCB's. Although more than a few contradictory information existing concerning the CCB's role on IOP [4,5,6] but the overall propensity is in the direction of a reduction in IOP [7,8,9]. Potential applications are reported about CCBs for their role in glaucoma including vasodilatation and thus refining optic nerve blood flow and neuro protection [10]. The technique to increase IOP through using steroids (suspension) was as described by [11]. The current work done is envisioned to notice the usefulness of topical diltiazem on steroid persuaded elevated intraocular pressure in rabbits. The result of the study will lead to an addition in the existing conflicting data.

MATERIALS AND METHODS

It was an experimental study which was conceded on rabbits in two phases including phase-A& phase-B.

Phase-A: Duringthis phase, ocular hypertension was created in the animals of sets X & Y excepting the standard/control set Z. The phase was continued for 3 weeks i.e. twenty one days (range 0-21).

Phase-B: A two days gap was given before the start of phase-B, to acquire a completely established elevated IOP (day 22 & 23).

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Through phase B, animals of set X(made ocular hypertensive during phase A), were provided a treatment with topical diltiazem ($8.9x10^{-2}$ M) solution. Set Y was in still mock tears. 4 weeks i.e. 28 days (day 24-51) were consumed during this phase. Infusion of the drugs was a single drop throughout the week.

Animals Used: 40 rabbits were brought for the study. The experiment was conceded on both eyes of standard and cognizant rabbits. Animals of either sex (male and female) orspecies (albino and colored strains) were incorporated. Their average weight was between 1500–2000 gms and their age was in between 1-2 years. Two weeks observation was done before the onset of the experiment. The animals were retained in the "Animal House, Khyber Medical University, Peshawar". Feeding was done on fooder, wheat grains ad libitum Fresh and nutritious water was also provided.

Grouping of Animals: Rabbits were organized in three sets.

Set X: This set contained 10 rabbits which were steroid treated and ocular hypertensive. The animals of this set were infused with topical diltiazem (8.9×10^{-2} M for four weeks).

Set Y: 20 rabbits were included in this set. Ocular hypertension was created within this set of animals. The set worked for ocular hypertensive control. It got mock tears for a period of four weeks.

Set Z: 10 rabbits were retained within this set. It was aimed to serve as normal controlor normotensive. No treatment was given to this set of animals.

Chemicals: Various chemicals were used during the study including Diltiazem HCl powder, Proparacaine HCl 0.5%, Inj. Betamethasone suspension, Fluorescein sodium 2% and artificial tears drops.

Equipments: Tonometer and rabbits containers were used in the study.

Initiation of Glaucoma: 1. Set X & Y animals were made ocular hypertensive(n = 30). A sub conjunctival suspension of betamethasone (betamethasone sodium phosphate& betamethasone acetate 3mg/ml each)/week (0.7ml) was infused in both eyes.

2. Infusion of Betamethasone was given for three weeks

3. Injections were given at day zero, 1, 2 & 3.

Procedure for Injecting Beta Methasone: Specially manufactured wooden boxes were used to keep the rabbits within them. The rabbits were infused. 5% proparacaine HCl, to persuade local anesthesia, was used. Sooner after some time, betamethasone was administered in sub conjunctival pouch of the animals Insulin injects were incorporated to achieve the same.

Procedure of Determining IOP: 1.All the rabbits were tested for their IOP using tonometer for two weeks (Before the start of the study). Four readings were noted during this time. Animals showed variations more than 5mm Hg in their intraocular pressure were omitted(n = 5) and newer animal's set was involved to swap the omitted ones.

2. To evade diurnal difference of the IOP, readings were taken at a fix time during the entire study (Ocular Pharmacology Text Book 1997).

3. Measurements of the IOP in both eyes were taken twofoldin a week. Corneal epithelial damage was protected by doing this (Kanski 2004). Thursday and Monday were selected for these practices.

4. Through phase-A, 1st reading was noted prior to injecting weekly Betamethasone (Thursday) and 2nd was recorded after 3 days (Monday).

5. Base line pressure was considered after infusing 1st injection of Betamethasone. It was designated as "zero time".

6. Before taking readings, the animals were provided with topical local anesthesia followed by fluorescein that causesstain in cornea.

7. Animals immobilization was done by placing them in specially designed wooden boxes.

8. IOP was recorded with the help of tonometer.

9. In phase-B, steroids infusion was ceased but IOP measurement was still continued. IOP was noted prior to the infusion of the drugs.

10. 2nd phase IOP readings were well-thought-out to be the initial pressure.

Preparation of Diltiazem: The only available form of diltiazem isthe tablet form.. Its ophthalmic solution is not readily available.

A strength of 8.9×10^{-2}M is known to possess intraocular pressure lowering effects (Juan Santafe 1997). We took the same solution and preceded our work.

Process for Drug Therapy: 1. Infusion of diltiazem & artificial tears was remained in progress during 2ndphase (day 24th).

2. It was completed in both eyes at a specific time.

3. Readings of the IOP was noted down prior to the infusing the drug.

RESULTS

Readings (IOP) were taken. Similar readings of both eyes were observed. (Right eye readings mentioned only). The readings shown as *, ** and NS

*= Significant (P<0.05), ** = Highly significant (P<0.05), **NS**= Non significant (P>0.05)

Table No.1: Set Y and Zmean IOP during 1st and 2ndphase

Time Interval (Weeks)	**Set Y** (Ocular hypertensive)	**Set Z** (Normotensive control)
0	19.63±0.64	20.00 ±0.31
1	22.04±0.65*	21.63± 0.60
2	22.65±0.25**	21.60±0.30
3	24.02±0.58**	22.86±0.46
4	24.64±0.24**	21.84±0.46
5	26.56±0.33**	21.80±0.56
6	26.60±0.26**	22.02±0.52
7	26.45±0.40**	22.05±0.50
8	25.45±0.30**	21.09±0.40

Table No. 2: Sets X&Y mean IOP differences during 2nd phase

Time Interval (Weeks)	Set X (Topical diltiazem)	Set Y (Artificial tears)
0	25.36± 0.30	25.50± 0.22
1	25.55 ±0.26**	26.54 ±0.32
2	21.29 ±0.70**	26.55± 0.25
3	21.04 ±0.80**	26.40± 0.42
4	20.52 ±0.64**	25.38 ±0.32

Table No.3: Week wise mean IOP difference of diltiazem treated ocular hypertensive rabbits

NB: 2nd measurements of IOP has been mentioned only

	Time Interval (Weeks)	Set X (Diltiazem treated)	Mean difference
0	Starting IOP	25.40±0.32	0.91±0.22
	Week 1 / Value 2	24.40±0.26	
1	Week 1 / Value 2	25.43±0.26	3.20±0.60**
	Week 2 / Value 2	20.26±0.70	
2	Week 2 / Value 2	20.24±0.71	0.30±0.40**NS**
	Week 3 / Value 2	20.00±0.78	
3	Week 3 / Value2	20.20±0.79	0.51±0.60*
	Week 4 / Value 2	19.45±0.60	

DISCUSSION

CCB's are known to be under use for more or less than 30 years for their IOP sinking properties. Sufficient facts are accessible regarding IOP lowering potency of ccbs. Its effects aretestified in man as well as in animals. Contradictory reports are there but still, there is no consensus on the sames[12-15].

Above all, even then, CCB's are considered to be important for the researchers due to their probable effect in glaucoma patients in lowering IOP as well as providing vasodilatation and neuro protection[16-19]. AGS (American Glaucoma Society) has connectedthe usage of iron and calcium augmentation in glaucoma victims (22nd annual meeting)[20].

The aforesaid work was conceived to validate IOP dropping tendency of diltiazem topically. The results substantiates that diltiazem can lesser intraocular pressure, consequently, resultingin an ad in the current information which shows CCB's role in managing glaucoma/ocular hypertension.

Steroid headed to a fast increase in IOP of setX & Y. The improvement in IOP was established statistically momentous after 2nddose of betamethasone showing a P value <0.05 as shown in table 1. After 4th injection, the progression became extremely significant statistically(P<0.05).

The normotensive control set Z, did not display any statistically noteworthy alteration in their IOP'S throughout the work done (P>0.05).

After interpreting the results of phase-B, it was noticed that topically smeared diltiazem abridged the IOP efficiently as shown in table 2. Set X result are highly statistically significant (P<0.00) in comparison with the control setZ (ocular hypertensive).

Looking at table 2, the alteration in IOP of set X as compared to set Y became highly significant statistically from the first7 days of treatment (P<0.00). Topical diltiazem substantiated to be effective in sinking IOP. Topical diltiazem has the tendency to drop the IOP very energetically, predominantly between 1st and 2nd week. A constant level in IOP was observed between 3rd and 4th week. Adiredrip in the IOP noticed was 5.10±0.61 between week 0 and 2. In the last week of treatment, it showed statistically non-significance (P>0.05), when its IOP lowering effect was compared week wise (Table 3).

After steroids cessation, some natural IOP lowering effect was also seen in set Y. The IOP dip was found significant statistically(P>0.05)in comparison with the values perceived at the termination of betamethasone therapy (Week 3). After termination of steroids treatment, the IOP was checked for additional 4 weeks in both sets.

This study does not shelter mechanism of action of diltiazem & any related drug related harmfulness except steroids. In conclusion, we recommend additional laboratory and animal models studies to discover its IOP sinking action and validate any systemic or local untoward effects. Diltiazem should be tested in human volunteers and then in glaucoma/ocular hypertension patients. Topical diltiazem effect on vasodilatation and nerve protection obviously needs more high profile research work.

CONCLUSION

It is evident that topical diltiazem is helpful to treat acute ocular hypertensive crisis because of its quick IOP droppingproperties, contributing to a reduction in glaucoma related morbidity and economic costs. Dose adjustment must be mandatory on individual basis.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Kanellopoulos J, Kristine AE, Peter AN. Calcium Channel Blockers and Glaucoma. Laser Vision.gr 2005
2. Allingham RR, Damji and Shield MB. Shield's Text Book of Glaucoma. 6th ed. McGraw Hill USA;2010.p.134-46
3. Kole P, Bhusari SS, Bhosale SM Kundu S, Gunasekaran J, Kaushal S, et al. Exploring Therapeutic Utilities of Calcium Channel Blockers. Pharmabiz.com 2009
4. Kelly SP, Wally TJ. Effect of Calcium Antagonist Nifedipine on Intraocular Pressure In Normal Subjects. British J. Ophthalmol 1998; 72: 216-18
5. Payne LJ, Slage TM, Cheeks LT, Green K. Effect of Calcium Channel Blockers on Intraocular Pressure. Ophthalmic Res 1990; 22(6): 337-41.

6. Liu S, Araujo SV, Spaeth GL, Katz LJ, Smith M. Lack of Effect of Calcium Channel Blockers On Open Angle Glaucoma. Glaucoma 1996;5(3): 187-90
7. Segarra J, Santafe J, Garrido M, Martinez de Ibarreta MJ. The Topical Application Of Verapamil And Nifedipine Lowers IOP In Conscious Rabbits. Gen Pharmacol 1993;24(5): 1163-71
8. Peter AN, Neena C,Evan BD. Calcium Channel Blockers in the Management of Low Tension and Open Angle Glaucoma. Am. J. Ophthalmol 1993; 115: 608-13
9. LukschA, Rainer G, Koyuncu D, Ehrlich P,Maca T, Schwandtner ME, Vass C,Schmetterer L. Effect of Nimodipine on Ocular Blood Flow and Colour Contrast Sensitivity in Patients with Normal Tension Glaucoma. British. J.Ophthalmol 2005; 89: 21-25
10. Ozlam EA, Karanjitt SK. Future Role of Neuroprotective Agents in Glaucoma. In: Thomas J Zimmerman, Karanjitt S Kooner, MordechaiShavir, Robert D Fechtner. Text Book of Ocular Pharmacology. 2nd Ed Philadelphia. Lippincott-Raven Publisher USA; 1997: 329-47
11. Juan S, Martinez de,Irreta MJ, Segarra J, Melena J. A Long Lasting Hypotensive Effect of Topical Diltiazem On The IOP in Conscious Rabbits. Naunynschmiedebergs Arch Pharmacol 1997; 355(5):645-50
12. Beatty JF, Krupin T, Nichols PF, Becker B. Elevation of Intraocular Pressure by Calcium Channel Blockers. Arch Ophthalmol 1984; 102(7); 1072-6
13. SP Kelly and TJ Wally. Effect Of Calcium Antagonist Nifedipine On Intraocular Pressure In Normal Subjects. Bri J Ophthalmol 1998; 72: 216-18
14. Payne LJ, Slage TM, Cheeks LT, Green K. Effect of Calcium Channel Blockers on Intraocular Pressure. Ophthalmic Res 1990; 22(6): 337-41
15. Liu S, Araujo SV, Spaeth GL, Katz LJ, Smith M. Lack of Effect of Calcium Channel Blockers On Open Angle Glaucoma. Glaucoma 1996;5(3): 187-90
16. Koseki N, Araie M, Tomidokoro A, Nagahara M, Hasegawa T, Tamaki Y and Yamamotos. A Placebo-controlled 03 Years Study of Calcium Blockers on Visual Field and Ocular Circulation in Glaucoma with Low-normal Pressure. Ophthalmol 2008;115(11): 2049-57.
17. Araie M, Mayama C. Use of Calcium Channel Blockers for Glaucoma. ProgRetin Eye Res 2011; 30(1):57-71.
18. Chihiro M. Calcium Channels and Their Blockers in IOP and Glaucoma. Eur J Pharmacol 2013
19. Anastasios J Kanellopoulos, Kristine A Erickson and Peter A Netland. Systemic Calcium Channel Blockers and Glaucoma. Laser Vision 2014.
20. Calcium/ Iron Supplementation and Glaucoma Linked. American Glaucoma Society 22nd Annual Meeting 2012.