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EVALUATION OF OCULOHYPOTENSIVE EFFECT OF ENALAPRILAT USING INTRAOCULAR PRESSURE (IOP) RECOVERY MODEL IN RABBITS

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ABSTRACT: The present study was designed to evaluate the oculohypotensive effect of enalaprilat in comparison to timolol using an intraocular pressure (IOP) recovery model. The study was carried out in 18 adult albino rabbits of 2.5-3.0 kg which were divided into 3 groups. Group I received normal saline, group II received enalaprilat (0.1%) and timolol (0.5%) was administered in group III. IOP recovery model was used to assess the effect of drugs on IOP recovery time. Additionally relative percent of IOP (IOP_t%) at various time intervals was calculated by equation: $IOP_t\% = (IOP_t / IOP_{-40}) \times 100$ % . Condition of conjunctiva was also examined. There was a significant (p= 0.006) delay of the IOP recovery with both timolol (150 ± 8.65 minutes) and enalaprilat (130 ± 6.83 minutes) when compared to normal saline (110 ± 6.83 minutes). There was also a change in relative percent of IOP at all the time intervals being significant at 80 minutes (p=0.036), 100 (p=0.006) and at 120 minutes (p=0.032). There was no congestion of conjunctiva with both timolol and enalaprilat. The findings suggest that enalaprilat has an oculohypotensive action and it may prove to be an additional effective, safe and may be a cost-effective topical drug for the management of glaucoma.

INTRODUCTION: Glaucoma is the leading cause of irreversible blindness due to optic nerve damage and is usually associated with an increased intraocular pressure. Globally, there are an estimated 60 million people with glaucomatous optic neuropathy and an estimated 8.4 million people who are blind as a result of glaucoma [1]. The incidence of glaucoma is closely related to aging and is markedly increased in people over the age of 40 [2, 3].

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An optimal balance between aqueous production and outflow maintains IOP in a normal eye. Thus an increase in IOP can be attributed to either an increase in aqueous humor formation or decrease in outflow[4]. Ongoing research mainly targets aqueous humor production, outflow facility and prevention of damage to retinal ganglion cells [5]. Intraocular pressure (IOP) reduction forms the primary goal in glaucoma management. Though many classes of anti-glaucoma drugs such as beta-blockers and prostaglandin inhibitors are available but they have their own limitations, necessitating the need for newer drugs with an equal or improved efficacy and a better safety profile. Prostaglandin analogs which are commonly prescribed these days are associated with reduced patient compliance due to corneal hyperemia, iris pigmentation and high cost.

Beta-blockers, another class of drugs in the management of glaucoma exacerbate asthma and worsen congestive heart failure.

Use of topical carbonic anhydrase inhibitors has reportedly been associated with burning, punctate keratitis and ocular allergies. These drugs are contraindicated in patients with hypersensitivity to sulfa medications and severe renal impairment [5]. Thus, there is a huge therapeutic lacuna which needs to be filled with newer drugs which have an effective anti-glaucoma action, fewer side effects and are cost-effective.

The importance of RAS in regulating systemic blood pressure is well established. Angiotensin II (Ang II) by activating Ang-II receptor type I acts as a potent vasoconstrictor and regulates systemic blood pressure [6]. Recently, existence of angiotensin receptors has been demonstrated in the eyes of arterial hypertensive rats [7]. Active local intraocular renin-angiotensin system (RAS) has been shown to exist in the human eye and evidence is accumulating that antihypertensive drugs acting on RAS can lower the IOP [8].

Few reports suggest the existence of local RAS in eye. Wagner et al demonstrated the gene expression for various components of RAS in ocular tissues [9]. Gene expression for angiotensin receptors has been identified in several ocular tissues, including the retina [10].

Angiotensin II has been implicated in the pathogenesis of glaucoma by causing vasoconstriction and vascular remodeling. As recently discovered by Tikellis et al, ACE-2 can degrade Ang II to Ang (1-7) [11] which is a potent vasodilator and antiproliferative molecule. Ang (1-7) mainly acts through a new angiotensin receptor type, Mas receptor [12]. Anderson DR suggested role of angiotensin induced vascular tone in glaucomatous cupping and optic nerve damage [13].

ACE inhibitors by inhibiting RAS in ocular tissues result in vasodilatation which could be one of the major mechanisms responsible for the IOP reduction. Also these are expected to prevent remodeling thereby producing a neuroprotective action. Drugs inhibiting components of RAS have been studied in different animal models.

Lotti and Pawlowski studied the effect of enalaprilat in African Green monkeys and found it effective in lowering IOP [14]. Oculohypotensive effect of ACE inhibitors has also been revealed by Shah et al in both acute and chronic models of glaucoma who reported that prodrugs like enalapril and ramipril failed to produce any change in IOP however there was significant reduction in IOP with active form like enalaprilat [15].

Another study with perindopril has shown a reduction in IOP in experimentally induced acute and chronic glaucoma in rabbits [16]. Recently, topically instilled aliskiren, a renin inhibitor by blocking RAS, was found to decrease IOP in water overloaded animals [17]. In some animal studies, topical application of angiotensin receptor antagonists also has been found to reduce IOP [18]. Some human studies with drugs inhibiting RAS have also shown promising results in glaucoma. Orally administered losartan (angiotensin type 1 receptor blocker) [19] has been shown to have oculohypotensive action.

In view of the existing need for better anti-glaucoma therapy and potential of ACE inhibitors as efficacious agents in glaucoma therapy, this study was conducted with the aim of evaluating the oculohypotensive effect of enalaprilat and comparing it with that of timolol using the IOP recovery model.

MATERIALS AND METHODS: The study was conducted in the Department of Pharmacology, Pt. B. D. Sharma PGIMS, Rohtak after approval from the Institutional Animal Ethics Committee (IAEC).

Experimental Animals: Adult albino rabbits, of either gender, of weight 2.5-3.0 kg were used. They were given food and water *ad libitum*. The animals were maintained under standard conditions in the animal house facility approved by the Institutional Animal Ethics Committee (IAEC) and according to guidelines issued by Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA). The animals were provided with normal pellet diet and tap water *ad libitum*. All animals were examined and those found normal on general and ophthalmic examination were included in the study. After one week of habituation in animal house facility the animals were trained to accept tonometry.

Estimation of IOP: Animals were appropriately restrained and IOP was estimated using Schiotz tonometer. Topical lignocaine (2%) was used to produce corneal anaesthesia before recording IOP. Antibiotic drops were instilled after each recording to avoid the possibility of infection.

IOP recovery model: The evaluation of IOP lowering activity of the test drug was carried out using IOP recovery model as described by Chiang *et al* [20]. Adult white normotensive rabbits of 2.5-3.0 kg weight range were utilized. 10 ml of sterile hypertonic saline (10% sodium chloride solution) was infused through the marginal ear vein at the rate of 1 ml/min. The drug was instilled in the form of eye drops on to the cul de sac of the right eye (treated eye) and vehicle on to the left eye (control eye), immediately after the infusion of hypertonic saline. IOP was measured at 40 and 20 minutes prior to instilling the eye drops as baseline and then at 0, 20, 40, 60 and 80 minutes thereafter at 20 minutes interval till the baseline values were obtained. The relative percent of IOP (IOP_t%) at various time intervals in both control and test eyes were calculated by the following equation.

$$IOP_t\% = (IOP_t / IOP_{-40}) \times 100\ \%$$

Where IOP_t%= relative percent of IOP at time t.

After calculating the relative percent of IOP (IOP_t%), ΔIOP_t% was obtained where ΔIOP_t% is the difference of IOP_t% between treated and controlled eyes.

Study Drugs: Enalaprilat (0.1%) was used as test drug. Timolol maleate (0.5%) was used as a reference standard.

Experimental Protocol: Eighteen normotensive rabbits were divided into three groups, with each group having six rabbits. The following medications were given after producing the IOP recovery model.

1. **Group I-** 50µl of normal saline (vehicle) topically instilled on to the *cul de sac* of both right eye (test eye) and left eye (control eye).
2. **Group II**- 50µl of enalaprilat (0.1%) topically instilled on to the cul de sac of right eye (test eye) and 50µl of normal saline on to the left eye (control eye).
3. **Group III**- 50µl of timolol (0.5%) topically on to the cul de sac of right eye (test eye) and 50µl of normal saline on to the left eye (control eye).

IOP was measured with the help of Schiotz tonometer at 40 and 20 minutes prior to instilling the eye drops, which served as baseline value and then at 0, 20, 40, 60 and 80 minutes thereafter at every 20 minutes interval till the baseline values were obtained.

Time taken for the recovery of IOP, the relative percent of IOP (IOP_t%) at different time intervals and ΔIOP_t% were noted and compared in all the three groups.

Statistical analysis: The data was recorded as Mean ± Standard Error of Mean (S.E.M) and was subjected to statistical analysis using one way ANOVA followed by post-hoc test. A 'p' value <0.05 was considered as statistically significant.

RESULTS:

Time taken for the recovery of IOP:

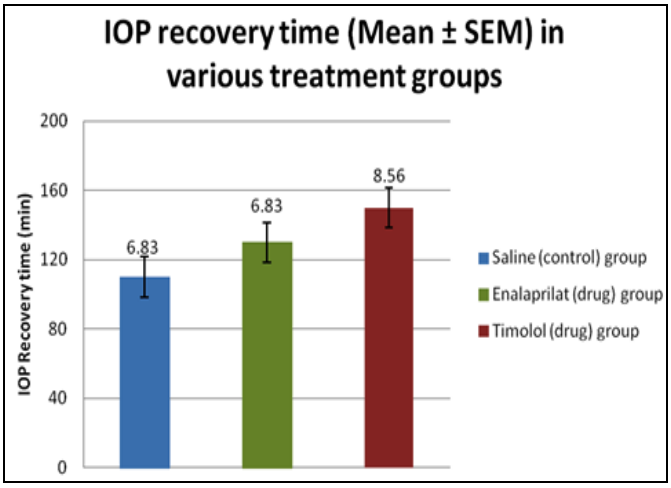

FIGURE 1: COMPARISON OF IOP RECOVERY TIME AMONG THE THREE TREATMENT GROUPS. Data shown as Mean ± SEM

There was an increase in the mean IOP recovery time in both the study groups receiving enalaprilat and timolol (p=0.006). The action of timolol on the IOP recovery time was superior to enalaprilat though the difference was not significant (p=0.174) when post-hoc analysis was done.

This delay in the time of IOP recovery by the test drugs is indicative of an oculohypotensive effect of both the study drugs and that timolol 0.5% is superior to enalaprilat 0.1% in this regard**.**

The relative percent of IOP (IOP_t%): The IOP_t% values with both the test drugs i.e. enalaprilat and timolol were compared to the baseline values at -40 minutes i.e. 40 minutes before the drug administration. The maximum decrease in IOP_t% was seen at 0 minutes after infusion of hypertonic saline in all the three groups with a subsequent rise in the values at various time intervals. In the saline group the IOP_t% at 0 minutes was 63.92 ± 2.25 and gradually increased to attain the baseline values at 140 minutes. In the enalaprilat group, IOP_t% at 0 minutes was 64.09 ± 1.51 and reached baseline values at 160 minutes. Similarly in timolol group, the baseline values were obtained at 180 minutes. (**Figure. 2**)

These findings indicate that both enalaprilat and timolol not only delay the IOP recovery time but also there is lowering of the IOP_t% indicative of oculohypotensive effect of both these drugs.

IOP_t% (Mean ± SEM) at Different Time Intervals is shown in **Table 1.**

TABLE 1: IOPt% AT DIFFERENT TIME INTERVALS

Time (min)	IOPt% (Mean ± SEM)			
	Saline group	Enalaprilat group	Timolol Group	P Value
-40	100 ± 0.00	100 ± 0.00	100 ± 0.00	0.00
-20	100 ± 0.00	101.33 ± 1.33	100 ± 0.00	0.391
0	63.92 ± 2.25	64.09 ± 1.51	60.29 ± 2.19	0.349
20	69.64 ± 1.02	70.37 ± 2.26	67.17 ± 3.66	0.657
40	74.16 ± 1.46	74.74 ± 2.63	70.33 ± 4.67	0.585
60	81.95 ± 1.50	76.95 ± 2.40	74.47 ± 4.74	0.271
80	87.98 ± 1.75	80.40 ± 2.29	76.58 ± 3.98	0.036*
100	97.21 ± 1.76	89.17 ± 2.34	82.18 ± 3.83	0.006*
120	98.58 ± 1.41	95.90 ± 2.72	88.15 ± 3.26	0.032*
140	100 ± 0.00	98.65 ± 1.35	94.50 ± 2.70	0.100
160	100 ± 0.00	100 ± 0.00	98.62 ± 1.37	0.391
180	100 ± 0.00	100 ± 0.00	100 ± 0.00	0.00

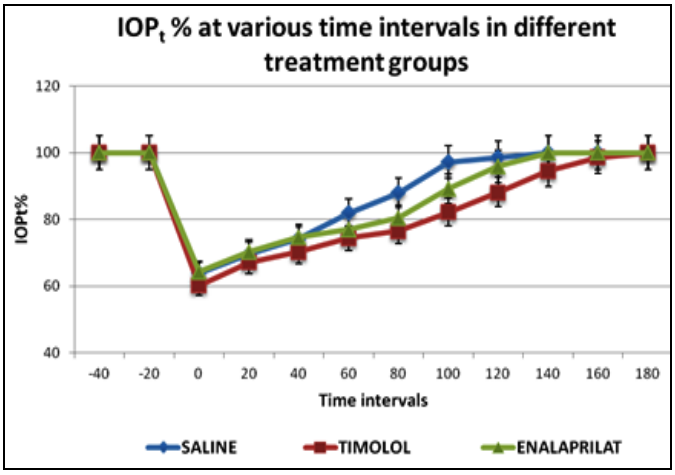

FIGURE 2: COMPARISON OF RELATIVE PERCENT OF IOP (IOP_t%) AT VARIOUS TIME INTERVALS IN DIFFERENT TREATMENT GROUPS

DISCUSSION: In this study, anti-glaucoma potential of enalaprilat was assessed using Intraocular Pressure (IOP) recovery model. This model is a simple, well documented and safe model not associated with any morbidity to the animal.

Our study is peculiar as this model has not been utilized in any of the studies on ACE inhibitors to the best of our knowledge. IOP recovery model is based on the recovery of IOP after a fall is induced by injecting hypertonic saline. Normally, this IOP returns back to the baseline value in a period of about 2 hours. A drug with a potential anti-glaucoma activity is expected to delay this recovery time. Chiang *et al* has used IOP recovery model in animal experiments to assess effect of certain drugs on intraocular pressure [20]. This model is not associated with any long term sequelae or morbidity.

Topical application of both enalaprilat and timolol delayed the IOP recovery. The observation is suggestive of an ocular hypotensive action with enalaprilat like that of timolol. The concentration of topical drugs used was 0.5% for timolol while for enalaprilat was 0.1%.

A higher concentration of enalaprilat may probably produce an ocular hypotensive action equal to or may be better than timolol.

A number of other studies have also shown a decreased IOP with ACE inhibitors in various glaucoma models. Lotti and Powlowski observed that 0.5% enalaprilat significantly decreased IOP in African green monkeys [14]. ACE inhibitors produced a significant ocular hypotensive effect in acute and chronic models of glaucoma as revealed by Shah *et al* [15].

With regard to mechanism of action of ACE inhibitors, a number of hypotheses have been put forward. Lotti and Pawlowski observed that on topical administration of ACE inhibitors, ocular bradykinin levels were found to be higher which resulted in an increase in the prostaglandin synthesis. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and many other prostaglandin analogues are known to reduce the IOP significantly by increasing the uveoscleral outflow. Indomethacin by blocking prostaglandin synthesis attenuates the IOP lowering effect thus confirming the role of prostaglandins in reduction of IOP [14]. In addition to this, ACE inhibitors by preventing angiotensin II mediated vasoconstriction and remodeling also exert a neuroprotective action which is an important current strategy to halt the glaucoma pathological process.

CONCLUSION: In conclusion, enalaprilat an ACE inhibitor can produce an oculohypotensive action and thus be a useful, safe and possibly cost effective alternative topical agent in the management of glaucoma. However, more studies with the use of higher concentration of enalaprilat may be more revealing of its ocular hypotensive effect and its therapeutic utility as a topical antiglaucoma drug.

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