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Mechanisms of angiotensin converting enzyme inhibitor-induced IOP reduction in normotensive rats

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ABSTRACT

Angiotensin converting enzyme inhibitors (ACEIs) have been shown to lower intraocular pressure (IOP). Since, the ACEIs cause increased tissue prostaglandin levels, we hypothesized that the mechanisms of ACEI-induced IOP reduction have similarity with those of prostaglandin analogs. The present study investigated the involvement of matrix metalloproteinases (MMPs) and cytokine activity modulation as the underlying mechanisms of ACEI-induced ocular hypotension. The IOP lowering effect of single drop of enalaprilat dehydrate 1% was evaluated in rats pretreated with a broad spectrum MMP inhibitor or a cytokine inhibitor. Effect of angiotensin receptor blocker, losartan potassium 2%, was also studied to evaluate involvement of angiotensin II receptor type 1 (AT_1) in IOP lowering effect of ACEI. Topical treatment with single drop of enalaprilat resulted in significant IOP reduction in treated eye with mean peak reduction 20.3% at 3 h post-instillation. Treatment with losartan resulted in a peak IOP reduction of 13.3%, which was significantly lower than enalaprilat, indicating involvement of mechanisms in addition to AT_1 blockade. Pretreatment with a broad spectrum MMP inhibitor or a cytokine inhibitor significantly attenuated the enalprilat-induced IOP reduction with mean peak IOP reduction of 11.2% and 13.6% respectively. The IOP-lowering effect of enalaprilat seems to be attributed to reduced angiotensin II type 1 receptor stimulation and modulation of MMP and cytokines activities.

1. Introduction

Glaucomatous neuropathy is the most common cause of irreversible blindness worldwide and is characterized by loss of retinal ganglion cells (Kuehn et al., 2005). Elevated IOP is a well-recognized risk factor associated with glaucoma (Tielsch et al., 1991) and, currently, is the only pharmacological target for therapeutic intervention in the management of glaucoma. The balance between aqueous humor production and its outflow is crucial for maintaining the normal IOP. Over-production of aqueous humor and/or impaired aqueous humor drainage leads to elevated IOP (Murgatroyd and Bembridge, 1998). Currently available antiglaucoma medications either reduce the rate of aqueous humor production or/and enhance its drainage and, thereby, primarily act by reducing the IOP. Existing therapies that are in clinical practice include sympathomimetics, β-blockers, miotics, prostaglandin analogs and carbonic anhydrase inhibitors. Several others that are under investigation include cytoskeleton agents, siRNAs, cannabinoids, ligands for serotonin, dopamine and adenosine receptors and many others (Bucolo et al., 2013). These agents often provide suboptimal IOP lowering and are associated with local and systemic adverse effects. Therefore, search for newer agents that can effectively lower IOP with minimal adverse effects continues to be an important area of investigation.

The components of renin-angiotensin system have been detected in ocular tissue (Cullinane et al., 2002; Van Haeringen, 1996) and therefore, ACE inhibitors although have shown efficacy in animal studies, the same in human is not established. Topical instillation of enalapril 0.01% in rabbits and 0.05% in African green monkeys have been shown to lower IOP (Lotti and Pawlowski, 1990; Watkins et al., 1987). The mechanisms underlying the ACE inhibitors-induced ocular hypotension have not been investigated widely, although, it has been proposed that IOP lowering effect of ACE inhibitors is mediated through reduced activation of AT1 receptors secondary to inhibition of angiotensin II production. However, ACE inhibitors also cause accumulation of bradykinin by

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preventing its breakdown. Since, bradykinin is a potent stimulant for prostaglandin synthesis (Fletcher et al., 2010) and pretreatment with indomethacin has been shown to inhibit the IOP lowering effect of ACE inhibitors (Shah et al., 2000), the mechanisms of ACE inhibitors-induced IOP lowering are expected to resemble those of prostaglandin analogs that are currently used in the treatment of glaucoma. The prostaglandin $F_{2\alpha}$ agonists have been shown to reduce IOP by modulation of MMPs and tumor necrosis factor-alpha (TNF-α) in the outflow pathways (Husain et al., 2008).

The present study for the first time has investigated possible modulation of MMPs and cytokine activity as the underlying mechanisms in the ACE inhibitors-induced ocular hypotension. We observed the effects of enalaprilat on IOP lowering in the presence GM 6001 (N-[(2R)-2-(hydroxamidocarbonylmethyl)-4-methylpentanoyl]-L-tryptophan methylamide) and thalidomide. GM 6001 is a wide spectrum MMP inhibitor (MMP-1,-2, -3, and -9 isotypes) and has been used widely as an MMP inhibitor in several studies (Bendeck et al., 1996; Cheng et al., 2009; Wang et al., 2006). We chose a wide spectrum inhibitor rather than a MMP subtype-specific inhibitor for the reason that prostaglandin analogs such as latanoprost have been shown to stimulate a wide range of MMPs (Oh et al., 2006; Weinreb and Lindsey, 2002; Husain et al., 2005). Since we hypothesized that mechanisms of ACEI-induced ocular hypotension have similarities with that of prostaglandin $F_{2\alpha}$ agonists, it was appropriate to study the IOP lowering effects of ACE inhibitor in the presence of wide spectrum MMP blockade. For the same reason we had chosen a wide spectrum cytokine inhibitor, thalidomide, which has been shown to inhibit expression of TNF-α and interleukins 6, 10 and 12 (Moreira et al., 1997; Rowland et al., 1998). Since, studies have shown that in vitro cell stimulation specifically by TNF-α is suppressed by thalidomide and thalidomide suppresses TNF-α to a greater extent than interleukins (Makonkawkeyoon et al., 1993; Bauditz et al., 2002); its cytokine inhibitory activity may be considered particularly, though not specifically, directed towards TNF-α. Thalidomide has been used previously to investigate TNF-α mediated effects of drugs (Gee et al., 2003; Husain et al., 2008).

2. Materials and methods

2.1. Animals

Male *Sprague Dawley* rats (200–300 g) were housed under standard laboratory conditions of 12 h cycles of light and dark and were provided with normal pellet diet with water ad libitum. After one week of acclimatization in the animal care unit, the animals were trained to accept tonometry. All procedures in this study were in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

2.2. Drugs

Enalaprilat dihydrate (ACE inhibitor), thalidomide, GM-6001, and losartan potassium (angiotenisn II type 1 receptor blocker) were purchased from Sigma-Aldrich Co LLC. Enalaprilat dihydrate and losartan potassium are water soluble and hence were constituted in a concentration of 1% and 2% respectively in hydroxypropyl methylcellulose 1% (HPMC). The selection of the concentration of enalaprilat and losartan was based on previous studies (Loftsson et al., 2010; Mehta et al., 2010; Inoue et al., 2001; Wang et al., 2005). Thalidomide 5% and GM-6001 0.05% were prepared in saline containing dimethyl sulfoxide (DMSO) 10% due to the lipophilic nature of drugs as described previously (Husain et al., 2008).

2.3. Study design

2.3.1. Study 1

2.3.1.1. Grouping and treatment. Forty *Sprague Dawley* rats were randomly divided into 4 groups of 10 animals each. All animals received topical bilateral pretreatment as per their respective groups. We included group 1 that provided a control to eliminate effects due to DMSO-induced changes in corneal permeability in treated groups, if any. All topical pretreatment was given as 10 μL eye drop applied twice at an interval of 15 min. One hour later, one of the randomly chosen eyes was treated topically, with 10 μL of the drug (treated eye) according to respective groups and the same volume of corresponding vehicle (HPMC 1%) was instilled in the contralateral eye (control eye).

Group 1: Pretreatment with DMSO 10%, followed by enalaprilat 1% in treated eye.
Group 2: Pretreatment with GM 6001 0.05% followed by enalaprilat 1% in treated eye.
Group 3: Pretreatment with thalidomide 0.5% followed by enalaprilat 1% in treated eye.
Group 4: Pretreatment with DMSO 10%, followed by losartan potassium 2% in treated eye.

2.3.1.2. IOP estimation. IOP estimations were done using Tonopen XL in conscious animals after topical application of proparacaine. All estimations were done under the same diurnal lighting cycle. IOP was estimated at baseline ($t=0$ h) and subsequently at 1, 2, 3, 4, 5, 6, 8, 12, and 24 h post-treatment. All IOP estimations were done by two independent blinded observers. At each time point 10 estimations were done by each observer and mean of 10 was considered as single observation. Subsequently mean of two observers at each time point was taken as final observation.

2.3.2. Study 2

2.3.2.1. Grouping and treatment. Twenty *Sprague Dawley* rats were randomized into 4 groups of 5 animals each. Group 4 was included to eliminate effects due to DMSO-induced changes, if any. All rats received twice daily bilateral pretreatment at 7.00 AM and 7.00 PM followed by bilateral treatment with drug/vehicle according to their respective groups as in study 1, for a period of 1 week. Observations were made to assess for the signs of local toxicity such as redness, edema and lacrimation affecting cornea, conjunctiva and lids.

1. Group 1: Pretreatment with DMSO 10%, followed by enalaprilat 1%.
2. Group 2: Pretreatment with GM 6001 0.05% followed by enalaprilat 1%.
3. Group 3: Pretreatment with thalidomide 0.5% followed by enalaprilat 1%.
4. Group 4: Pretreatment with DMSO 10% followed by treatment with HPMC 1%.

2.3.2.2. Aqueous humor collection for prostaglandin estimation. At the end of 1 week treatment period, all animals were anesthetized by an intraperitoneal injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg). The ocular surface was washed with 0.9% saline and dried using soft filter paper. The central cornea was punctured shallowly using a 30 G needle, avoiding damage to the iris and compromise of the blood-aqueous barrier. Aqueous humor exited passively and was collected with a capillary tube. Aqueous humor specimens were collected in siliconized microfuge tubes