Research Paper

In vitro prevention by ACE inhibitors of cataract induced by glucose

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ABSTRACT

Objectives: To study, the anticataract activity of lisinopril and enalapril on cataract induced by glucose, in goat lenses.

Materials and Methods: Goat lenses were incubated in artificial aqueous humor containing 55 mM glucose (cataractogenesis) with lisinopril or enalapril in different concentrations at room temperature for 72 h. Biochemical parameters studied in the lens were electrolytes (Na^+, K^+), Na^+-K^+-ATPase activity, malondialdehyde (MDA) and proteins.

Results: Glucose induced opacification of goat lens began 8-10 hrs after incubation and was complete in 72-80 hrs. Cataractous lenses showed higher Na^+, MDA ($P<0.001$), lower Na^+-K^+-ATPase activity, and water-soluble protein content. Lenses treated with lisinopril or enalapril in concentrations of 1, 5, and 10 ng/ml showed higher protein (total and water soluble proteins) content and prevented formation and progress of cataract by glucose, as evidenced by biochemical parameters.

Conclusion: The anticataract activity of lisinopril and enalapril may be because of the antioxidant and free radical scavenging activity, as evidenced by a decrease in MDA in treated lenses. Further *in-vitro* and *in-vivo* studies in various experimental models and long term clinical trials are required to validate the anticataract activity of ACE-inhibitors.

KEY WORDS: Antioxidant, enalapril, lisinopril.

Introduction

Cataract is the opacification of lens often associated with old age and is a major complication of diabetes mellitus because higher glycosylated hemoglobin levels are significantly associated with increased risk of cataract.[1] Although many cataractogenic factors have been identified, the biochemical background of cataractogenesis is still unknown. The lens Na^+-K^+-ATPase activity plays an important role in maintaining lens transparency, and its alteration is one of the major events leading to the cataract formation.[2] Oxidative damage by the free radicals is also implicated in the pathology of cataractogenesis.[3] Although a number of agents have been tried for prevention and therapy of cataract, none have proved useful.[4] ACE inhibitors have been found to afford protection from free radical damage in many experimental conditions.[5]-[8] Therefore, this study was conducted to find the efficacy of lisinopril and enalapril in the prevention of experimental cataract induced by glucose.

Materials and Methods

Various *in vivo* or *in vitro* experimental models in rats, mice, and rabbits have been utilised to study cataractogenesis. In this study, goat lenses were used as they were easily available. The study was approved by the institutional ethics committee.

Lens culture

Fresh goat eyeballs were obtained from slaughterhouse immediately after slaughter and transported to the laboratory at 0-4 degree Celsius. The lenses were removed by extracapsular extraction and incubated in artificial aqueous humor (NaCl 140 mM, KCl 5 mM, $MgCl_2$ 2 mM, $NaHCO_3$ 0.5 mM, NaH $(PO4)_2$ 0.5 mM, $CaCl_2$ 0.4 mM and Glucose 5.5 mM) at room temperature and pH 7.8 for 72 h.[9] Penicillin 32 mg% and streptomycin 250 mg% were added to the culture media to prevent bacterial contamination. Glucose in a concentration of 55 mM was used to induce cataract.[9] At high concentrations, glucose in the lens was metabolised through sorbitol pathway and accumulation of polyols (sugar alcohols), causing overhydration and oxidative stress. This led to cataractogenesis.

Study drugs and groups

Lisinopril, enalapril, and captopril have been reported to possess free radical scavenging activity. Lisinopril and enalapril are used commonly in clinical practice, therefore, lisinopril and enalapril are used for this study. EC_{50} & EC_{90} concentrations for ACE inhibition with lisinopril are 5 - 20 and 27 ng/ml,

respectively, in humans after oral administration. Data on the concentrations of lisinopril and enalapril in ocular tissues after oral administration is not available. As this is a first study using ACE inhibitors, we planned to use concentrations of lisinopril and enalapril ranging from 1 to 10 ng/ml, anticipating these concentrations in ocular tissues in therapeutic doses used for cardiovascular conditions.

A total of 80 lenses were divided into following categories (n=10 in each category):

Group I : Normal lens [Control (Glucose 5.5mM)]
Group II : Glucose 55mM
Group III
A : Glucose 55 mM + Lisinopril 1 ng/ml
B : Glucose 55 mM + Lisinopril 5 ng/ml
C : Glucose 55 mM + Lisinopril 10 ng/ml
Group IV
A : Glucose 55 mM + Enalapril 1 ng/ml
B : Glucose 55 mM + Enalapril 5 ng/ml
C : Glucose 55 mM + Enalapril 10 ng/ml

Homogenate preparation

After 72 h of incubation, homogenate of lenses was prepared in Tris buffer (0.23M, pH 7.8) containing $0.25X10^{-3}$ M EDTA and homogenate adjusted to 10 % w/v.

The homogenate was centrifuged at 10,000 G at 4°C for 1 hour and the supernatant used for estimation of biochemical parameters. For estimation of water-soluble proteins, homogenate was prepared in sodium phosphate buffer (pH 7.4).

Biochemical estimation

Electrolyte (Na^+ & K^+) estimation was done by flame photometry. Na^+-K^+-ATPase activity was assessed by the method of *Unakar & Tsui* [10] and protein by *Lowry's method*.[11] The degree of oxidative stress was assessed by measuring the MDA levels by *Wilbur's method*.[12]

Photographic evaluation

Lenses were placed on a wired mesh with posterior surface touching the mesh, and the pattern of mesh (number of hexagons clearly visible through the lens) was observed through the lens as a measure of lens opacity.

Statistical analysis

All data were expressed as mean±SD. The groups were compared using one-way ANOVA with post-hoc Dunnett's test using glucose 55 mM group as control. $P<0.05$ was considered significant.

Results

Incubation of lenses with glucose 55 mM showed opacification starting after 8 hrs at the periphery, on the posterior surface of the lens. This progressively increased towards the centre, with complete opacification at the end of 72 hrs.

Biochemical changes

Glucose 55 mM treated lenses showed significantly higher Na^+ ($P<0.05$), lower K^+ ($P<0.001$) and lower Na^+-K^+-ATPase activity ($P<0.001$) compared with normal lenses. [Table 1] Both lisinopril and enalapril treated lenses, in concentrations of 1, 5, and 10 ng/ml showed significantly high K^+ ($P<0.001$), while Na^+-K^+-ATPase activity was significantly higher ($P<0.001$) with concentrations of 5 and 10 ng/ml, compared with glucose 55 mM alone group. [Table 1] Both ACE inhibitors treated groups showed a lower Na^+ compared with glucose 55 mM group at all three concentrations, but the difference was not statistically significant ($P>0.05$).

Glucose 55 mM treated lenses also showed significantly low concentrations of proteins (total and water soluble proteins) in the lens homogenate ($P<0.001$) and very high MDA ($P<0.001$) compared with control group having normal lenses. [Table 2] Both lisinopril and enalapril groups had significantly higher concentrations of total lens proteins at 5 and 10 ng/ml concentrations ($P<0.05$), compared with glucose 55 mM group. At the same time, they had higher water-soluble proteins at all three concentrations of lisinopril and enalapril, compared with glucose 55 mM group. However, the difference was not significant statistically. [Table 2]

MDA levels were found to be very high in glucose 55 mM treated lenses, compared with normal lenses (60.7 *Vs* 2.9). Lenses treated with lisinopril and enalapril had significantly reduced MDA content ($P<0.01$) at all the three concentrations, compared with glucose group. [Table 2]

Table 1

Na^+, K^+ and Na^+-K^+-ATPase activity in lens homogenate after 72 h of incubation

Study Groups		*Na^+[meq/gm]*	*K^+[meq/gm]*	*Na^+-K^+-ATPase activity [µgP/gm]*
Normal [Control]		153.7 ± 57.1*	10.5 ± 1.5***	41.8 ± 2.2***
Glucose 55 mM		209.7 ± 29.7	6.4 ± 0.3	17.7 ± 4.9
Glucose 55 mM + Lisinopril 1 ng/ml		182.5 ± 28.9	9.5 ± 2.2**	23.8 ± 9.8
Glucose 55 mM + Lisinopril 5 ng/ml		187.5 ± 29.4	9.2 ± 1.8*	35.5 ± 7.0***
Glucose 55 mM + Lisinopril 10 ng/ml		180.0 ± 40.4	10.6 ± 2.4***	35.4 ± 9.5***
Glucose 55 mM + Enalapril 1 ng/ml		177.5 ± 38.0	9.5 ± 2.5**	24.7 ± 10.7
Glucose 55 mM + Enalapril 5 ng/ml		180.0 ± 40.4	9.7 ± 2.7**	37.3 ± 5.5***
Glucose 55 mM + Enalapril 10 ng/ml		180.0 ± 43.7	9.2 ± 2.0*	32.9 ± 10.5***
One-way ANOVA	F	1.516	3.970	10.619
	df	7, 72	7, 72	7, 72
	P	0.176	0.001	<0.0001

Values are mean±SD. n=10 for each group. *P<0.05, **P<0.01 and ***P<0.001 as compared with their corresponding value in glucose 55 mM group.

Table 2

Proteins (total proteins and water soluble proteins) and malondialdehyde (MDA) in lens homogenate after 72 h of incubation

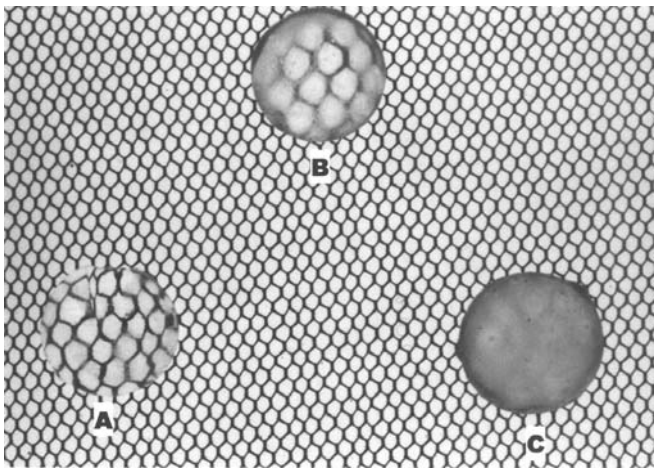
Study Groups		*Total proteins [mg/gm]*	*Water soluble proteins [mg/gm]*	*MDA [nmole/gm]*
Normal [Control]		226.8 ± 33.9***	94.1 ± 18.4**	2.9 ± 1.1***
Glucose 55 mM		160.8 ± 29.1	61.6 ± 29.4	60.7 ± 20.0
Glucose 55 mM + Lisinoril 1 ng/ml		165.8 ± 32.0	69.0 ± 23.8	42.8 ± 12.2**
Glucose 55 mM + Lisinoril 5 ng/ml		205.8 ± 29.0**	72.3 ± 21.7	32.5 ± 8.7***
Glucose 55 mM + Lisinoril 10 ng/ml		202.4 ± 17.1*	70.7 ± 25.4	24.3 ± 13.1***
Glucose 55 mM + Enalapril 1 ng/ml		187.4 ± 45.6	65.7 ± 28.0	37.37 ± 14.3***
Glucose 55 mM + Enalapril 5 ng/ml		214.9 ± 27.7***	65.7 ± 25.8	27.4 ± 7.4***
Glucose 55 mM + Enalapril 10 ng/ml		210.8 ± 23.5**	69.9 ± 29.3	27.9 ± 9.3***
One-way	F	6.265	2.151	22.041
ANOVA	df	7, 72	7, 72	7, 72
	P	<0.0001	0.049	<0.0001

Values are mean±SD. n=10 for each group. *P<0.05, **P<0.01 and ***P<0.001 as compared with their corresponding value in glucose 55 mM group.

Photographic evaluation [Figure-1]

After 72 h of incubation in glucose 55 mM, lens becomes completely opaque ('C') as against lenses incubated in 5.5 mM glucose ('A'). Incubation of lenses with lisinopril and enalapril, at all concentrations, seem to retard the progression of lens opacification, compared with control group (glucose 55 mM). This is because more number of hexagons are clearly visible in 'B' (Glucose 55 mM + Lisinopril 10 ng/ml) than in 'C' (Glucose 55 mM).

Figure 1. (A) Normal lens after 72 h of incubation (Transparency maintained, more hexagons clearly visible). (B) After 72 hours of incubation in glucose 55 mM + lisinopril 10 ng/ml, lens appears slightly hazy (less no. of hexagons clearly visible). (C) Complete cataractogenesis after 72 h of incubation in glucose 55 mM (Complete loss of transparency, no hexagons visible through lens).



Discussion

In cataractogenesis, the parameters commonly considered are electrolytes (Na^+ and K^+), malondialdehyde (MDA) and proteins (total proteins and water soluble proteins).

Incubation in the media containing high glucose (55 mM) concentration has shown to cause considerable drop in Na^+-K^+-ATPase activity, with progression of opacity.[10] This study, is in agreement with this finding. Na^+-K^+-ATPase is important in maintaining the ionic equilibrium in the lens, and its impairment causes accumulation of Na^+ and loss of K^+ with hydration and swelling of the lens fibers leading to cataractogenesis.[13] This alteration in the Na^+-K^+ ratio alters the protein content of the lens, leading to a decrease in water-soluble proteins' content and increase in insoluble proteins. This causes lens opacification.[14] This study showed higher Na^+-K^+-ATPase activity, total and water-soluble proteins and K^+ ions whereas lower concentrations of Na^+ ions with lisinopril and enalapril treated groups. Therefore, these ACE inhibitors seem to prevent the alteration of Na^+ and K^+ imbalance, which may be due to a direct effect on lens membrane Na^+-K^+-ATPase or indirect effect through their free radical scavenging activity.

Oxidative stress may also be implicated in the cataract induced by glucose, due to the formation of superoxide ($O_2^{\cdot-}$) radicals and H_2O_2. High glucose (55 mM) has shown to induce antioxidant enzymes, suggesting oxidative stress in the cells.[15] In this study MDA levels were significantly higher in high glucose (55 mM) group, compared with normal control group. The MDA levels were significantly less in the lisinopril and enalapril treated groups at all concentrations. These results are in agreement with those of Bhuyan KC, *et al*. [16] They found significant reduction in the rate of superoxide ($O_2^{\cdot-}$) production in animals treated with captopril, in cataract model induced by diquat in rabbits. Noda Y, *et al*.[17] demonstrated scavenging activity of lisinopril on nitric oxide. Lisinopril and enalapril have also been shown to increase the content of water-soluble proteins, retarding the process of cataractogenesis initiated by high glucose concentration.

Incubation in presence of high glucose (55 mM) concentration simulates a state of clinical diabetes where ACE inhibitors are commonly used in these patients to treat associated cardiovascular disorders. A preventive role of lisinopril and enalapril as seen in this *in vitro* model may, to some extent, suggest an additional utility of ACE inhibitors in the form of preventing and/or retarding the progression of diabetic cataracts.

The concentrations of lisinopril and enalapril used in this study ranged between 1 to 10 ng/ml. However, higher concentrations upto 20 ng/ml may show better anticataract activity, and further evaluation with higher concentrations is required. This *in vitro* study may not directly correlate with the *in vivo* conditions. Therefore, *in vivo* studies in different animal models are required for further elucidation of the role of ACE inhibitors in preventing cataract.

Conclusion

Lisinopril and enalapril demonstrated *in vitro* anticataract activity in experimental cataract induced by high glucose. Further *in vitro* and *in vivo* studies to elucidate the exact mechanism of ACE inhibitors in prevention of cataractogenesis are needed. Clinical evaluation of patients already receiving ACE inhibitors may be followed to identify the presence of the additional benefit of cataract prevention/progression.

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