International Journal of Pharmacy and Pharmaceutical Sciences, Vol. 1, Issue 2, Oct-Dec. 2009

Research Article

LISINOPRIL ATTENUATES SELENITE INDUCED EXPERIMENTAL CATARACT DEVELOPMENT: AN IN VITRO STUDY

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Received – 5th May, 2009, Revised and Accepted – 5th July 2009

ABSTRACT

Several studies have suggested that angiotensin converting enzyme inhibitors retard the process of cataractogenesis by scavenging free oxygen radicals. The present study sought to assess the efficacy of the ACE inhibitor Lisinopril in preventing selenite-induced cataractogenesis in an experimental setting. The *in vitro* phase of the study was performed on lenses from wistar rats incubated for 24 h at 37^0C in Dulbecco's Modified Eagle Medium (DMEM) alone (control, Group I), or in DMEM containing 100 µM of selenite (Group II) or in DMEM containing 100 µM of selenite and 10 µM lisinopril (Group III). Gross morphological examination of these lenses revealed dense opacification (cataract formation) in Group II, minimal opacification in Group III lenses and no opacification in Group I. The mean activities of the reduced glutathione, total protein and water soluble protein were significantly lower in Group II than in Group I or Group III lenses, while malondialdehyde concentration (an indicator of lipid peroxidation), insoluble protein and lens Ca^{2+} concentration was significantly higher in Group II lenses than that in Group I or Group III lenses. Reversal of these changes was found to be significant in lisinopril treated group of lenses. These data suggest that lisinopril is able to significantly retard experimental selenite-induced cataractogenesis.

Keywords: Cataractogenesis, ACE inhibitors, Lisinopril, Lipid peroxidation, Reduced glutathione

INTRODUCTION

Cataract is a multifactorial disease associated with several risk factors such as aging, diabetes, malnutrition, diarrhoea, sunlight, smoking, hypertension and renal failure[1]. Free radical-induced oxidative stress is postulated to be perhaps the major factor leading to senile cataract formation[2]. This hypothesis is supported by the anticataractogenic effect of various nutritional and physiological[3,4] antioxidants in experimental animals. Selenite cataract is a rapidly-induced, convenient model for the study of senile nuclear cataractogenesis. The morphological and biochemical characteristics of this model have been extensively investigated; moreover, this model shows a number of general similarities to human cataract. The reliability and extensive characterization of selenite cataract makes it a useful rodent model for rapid screening of potential anticataract agents[5]. Physiologic antioxidant such as pyruvate and nutritional antioxidant vitamin E, ascorbic acid and carotenoids were found to delay the experimental cataract. ACE inhibitors have been found to afford protection from free radical damage in many experimental conditions[6,7,8,9]. Selenite cataract, first described by Ostadalova et al in 1978, is an excellent model of oxidative stress-induced cataractogenesis *in vitro* and *in vivo*[10], hence it was used in the present study to evaluate the efficacy of lisinopril as a anticataractogenic agent.

MATERIAL AND METHODS

Chemicals

Lisinopril was kindly provided by Torrent Pharmaceutical Ltd (Ahmedabad, Gujrat, India) approximate purity was 98%. Dulbeco's modified Eagles medium (DMEM) was obtained from Hi Media Laboratories (Mumbai, India). Fetal bovine serums (FBS) and Sodium selenite purchased from Sigma Chemical Company, St. Louis, MO. 24 wells Falcon plastic culture plate was acquired from Genei, Bangalore India. All other chemicals and solvents were procured from SRL, Mumbai, India.

In vitro study

Wistar rats of either sex in the weight range 80 to100gm were used for the study. Rats used for the study were obtained from the animal house stock of the Department of Pharmacology, SRM College of Pharmacy, Kattankulathur, India and handle in accordance with the guidelines as per the "Institutional Animal Ethical Committee" and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) rules. Lenses were extracted through a posterior approach from the eyes of wistar strain rats under deep anesthesia. Lenses were organ cultured in DMEM medium with HEPES buffer, supplemented with 10% fetal calf serum (FCS), 100 U/ml enicillin, 0.1 mg/ml streptomycin, and 0.25 µg/ml amphotericin was also added to prevent bacterial contamination. Selenite medium was prepared by adding sodium selenite to the medium to give a final concentration of 0.1 mM. Lenses were maintained in a 24 well culture plate with 2 ml medium and Lenses were incubated for 24 hours under 5% CO_2 at 37°C in a CO_2 incubator. After 2 h of incubation, opaque lenses which are damage during dissection were discarded and transparent lenses were taken for the subsequent experimental studies.

Transparent lenses were divided equally into three different groups to serve as normal, control, and test groups. The lenses in the normal group were cultured in DMEM alone. The lenses in the control group were cultured in DMEM plus 100 µM sodium selenite, and those in the test group were cultured in the control medium plus 10 µM lisinopril. The dose of lisinopril was determined from previous study of Ghazi-Khansari et al., on liver mitochondrial cells culture study[11]. The stock solution (10 mM) of lisinopril was prepared in double distilled water by vigorous shaking and was clear to the unaided eye. All lenses were incubated for 24 h at the conditions described earlier. After incubation, lenses were processed for the estimation of biochemical parameters.

Reduced glutathione (GSH)

The GSH content was estimated by the method of Moron et al[12]. Half of the lenses from each group were weighed and homogenized in 1 ml of 5% trichloroacetic acid (TCA), and a clear supernatant was obtained by centrifugation at 5000 rpm for 15 min. To 0.5 ml of this supernatant, 4.0 ml of 0.3 M Na_2HPO_4 and 0.5 ml of 0.6 mM 5,5'-dithiobis-2-nitrobenzoic acid in 1% trisodium citrate was added in succession. The intensity of the resulting yellow color was read spectrophotometrically at 410 nm. Reduced GSH was used as a standard.

Estimation of malondialdehyde (MDA)

The extent of lipid peroxidation was determined by the method of Ohkawa et al[13]. Briefly, 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.81% thiobarbituric acid aqueous solution was added in succession. To this reaction mixture, 0.2 ml of the tissue

sample (lens homogenate prepared in 0.15 M Potassium chloride) was added. The mixture was then heated in boiling water for 60 min. After cooling to room temperature, 5 ml of butanol: pyridine (15:1 v/v) solution was added. The mixture was then centrifuged at 5000 rpm for 15 min. The upper organic layer was separated, and the intensity of the resulting pink colour was read at 532 nm. Tetramethoxypropane was used as an external standard. The level of lipid peroxide was expressed as nmoles of MDA formed in µmol/g wet weight for lenses.

Estimation of levels of Na^+, K^+ and Ca^{2+}

Electrolyte (Na^+, K^+ and Ca^{2+}) estimation was done by flame photometry and the results were expressed as %weight wet tissue. Standard stock solutions of cations were prepared by individually dissolving sodium chloride and potassium chloride in deionised water. Calcium carbonate was dissolved in 1 per cent nitric acid for the stock calcium standard solution.

Estimation of protein value

For total protein estimation the lens homogenate was prepared in 5% trichloroacetic acid. The precipitated protein was dissolved in Sodium hydroxide and used as aliquots for the estimation of total proteins. Soluble and insoluble fractions of the protein were estimated by preparing homogenate in double distilled water. The water soluble supernatant was used for estimation of soluble protein and the residue was dissolved in sodium hydroxide and used for the estimation of insoluble protein. The protein content of the samples was determined by the method of Lowry et al[14] using bovine serum albumin as the standard.

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 Selenite 100µM group as control. $P<0.05$ was considered significant.

RESULTS

Incubation of lenses with Selenite 100µM showed opacification starting after 2 hrs at the periphery, on the posterior surface of the lenses. This progressively increased towards the centre, with complete opacification at the end of 24 hrs. Selenite 100µM treated lenses showed significantly higher Na^+ ($P<0.01$) and Ca^{2+} ($P<0.01$) while lower K^+ ($P<0.01$) concentration compared with normal lenses. Lisinopril treated lenses showed significantly high K^+ ($P<0.01$), while Na^+ and Ca^{2+} concentration was significantly lower ($P<0.01$) compared with Selenite 100µM alone group (Table 1).

Table 1. Levels of lens Na^+, K^+ and Ca^{2+} in Group I, Group II and Group III

Parameter (%wt)	Group I	Group II	Group III
Sodium (Na^+)	0.196 ± 0.001*	0.315 ± 0.002	0.263 ± 0.004*
Potassium (K^+)	0.954 ± 0.002*	0.565 ± 0.002	0.896 ± 0.002*
Calcium (Ca^{2+})	0.014 ± 0.004*	0.025 ± 0.002	0.016 ± 0.001*

All values are expressed as mean±SD of five determinations.

Group I: Normal, Group II: lenses exposed to selenite only. Group III: lenses exposed to selenite and lisinopril.

Statistically significant difference ($^*P < 0.01$) when compared with group II values.

Selenite 100μM treated lenses also showed significantly low concentrations of proteins (total and water soluble proteins) in the lens homogenate (P<0.01) and very high insoluble protein (P<0.01) compared with normal group having normal lenses (Table 2). Lisinopril group had significantly higher concentrations of total lens proteins and water soluble protein (P<0.01), compared with Selenite 100μM group. At the same time, they had lower water-insoluble proteins (P<0.01) compared with Selenite 100μM group.

Table 2. Levels of total protein, water soluble protein and water insoluble protein in Group I, Group II and Group III lenses

Parameter (mg/mg wt.)	Group I	Group II	Group III
Total Protein	0.389 ± 0.003*	0.336 ± 0.012	0.368 ± 0.014*
Water Soluble Protein	0.282 ± 0.005*	0.180 ± 0.001	0.248 ± 0.004*
Water Insoluble	0.070 ± 0.002*	0.136 ± 0.001	0.102 ± 0.002*

All values are expressed as mean±SD of five determinations.
Group I: Normal, Group II: lenses exposed to selenite only. Group III: lenses exposed to selenite and lisinopril.

Statistically significant difference (*P < 0.01) when compared with group II values.

The mean GSH value in the normal lenses was 2.40±0.05 μg/mg of fresh weight of lens. A significant decrease in GSH level was observed in the presence of sodium selenite in the control as opposed to the normal group (P<0.01). In the presence of Lisinopril, there was a significant restoration of GSH level in the treated lenses (P<0.01) as opposed to the control lenses. The mean GSH values in the control and test groups were 1.36 ± 0.01 and 2.19 ± 0.01 μg/mg of fresh weight of lens, respectively. A significant increase in MDA level was found in the control opposed to the normal lenses (0.74 ± 0.02 μmol/g of fresh weight of lens; P<0.01). Lisinopril supplementation significantly protected (P<0.01) the test group lenses from lipid peroxidation; the MDA content was 0.067 ± 0. μmol/g of wet weight of lens (Table 3).

Table 3. Levels of reduced glutathione and malondialdehyde in Group I, Group II and Group III lenses

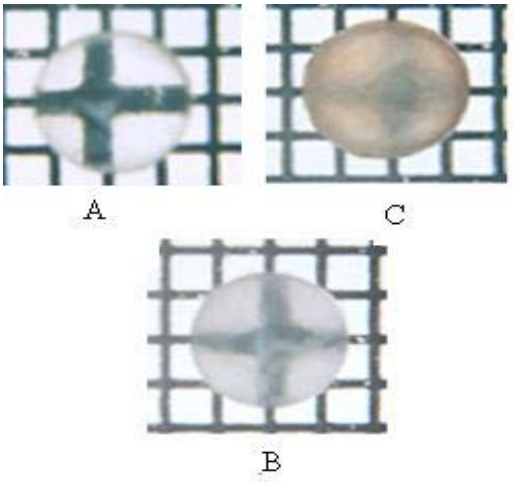
Parameter (μg/mg wt.)	Group I	Group II	Group III
GSH	2.40 ± 0.05*	1.36 ± 0.01	2.19 ± 0.01*
MDA	0.061 ± 0.001*	0.74 ± 0.02	0.067 ± 0.001*

All values are expressed as mean±SD of five determinations.
Group I: Normal, Group II: lenses exposed to selenite only. Group III: lenses exposed to selenite and lisinopril.
Statistically significant difference (*P < 0.01) when compared with group II values. GSH:glutathione; MDA:malondialdehyde.

Effect on morphology (Fig. 1)

After 24 h of incubation in Selenite 100 µM, lens becomes completely opaque ('C') as against lenses incubated in DMEM alone ('N'). Incubation of lenses with Lisinopril 10 µM, seem to retard the progression of lens opacification, compared with control group (Selenite 100 µM). This is because more number of hexagons are clearly visible in 'B' (Selenite 100 µM + Lisinopril 10 µM) than in 'C' (Selenite 100 µM).

Fig. 1: (A) Normal lens after 24 h of incubation (Transparency maintained, more hexagons clearly visible). (B) After 24 hours of incubation in Selenite 100µM + lisinopril 10 µM, lens appears slightly hazy (less no. of hexagons clearly visible). (C) Complete cataractogenesis after 24 h of incubation in Selenite 100µM (Complete loss of transparency, no hexagons visible through lens).



DISCUSSION

In cataractogenesis, the parameters commonly considered are electrolytes (Na^+, Ca^{2+} and K^+), malondialdehyde (MDA), reduced glutathione (GSH) and proteins (total proteins and water soluble proteins).

With regard to cataract, the selenite model was selected because of the rapid, effective and reproducible cataract formation. Although the rate of opacification in the selenite model is much more rapid than in human cataract, it has many general similarities to human cataract like increased calcium, protein aggregation, decreased water soluble proteins and level of reduced glutathione[5,15].

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[16]. 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[17]. This study showed higher total and water-soluble proteins and K^+ ions whereas lower water insoluble protein and Na^+ as well as Ca^{2+} ions concentration with lisinopril treated group. 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.

Chemical analysis of selenite treated lenses clearly demonstrated a significant depletion of GSH and increased membrane damage as indicated by the levels of MDA, the product of membrane lipid peroxidation. Such changes in GSH and MDA levels in presence of selenite have been reported[18]. Restoration of GSH and MDA levels, protection against aggregation and insolubilization of lens proteins, and maintenance of lens clarity without doubt establish the protective action of lisinopril.

These results are in agreement with those of Bhuyan KC et al[19]. They found significant reduction in the rate of superoxide (O_2^{-}) production in animals treated with captopril, in cataract model induced by diquat in rabbits. Noda Y et al[20] demonstrated scavenging activity of lisinopril on nitric oxide. Lisinopril have also been shown to increase the content of water-soluble proteins, retarding the process of cataractogenesis initiated by oxidative stress by sodium selenite.

In conclusion, we have demonstrated, for the first time, the prevention of selenite-induced cataractogenesis by lisinopril. This effect is associated with increased GSH, maintaining of protein level, mineral homeostatis and decreased MDA levels. These biochemical changes reiterate the important role of oxidative stress in selenite induced cataractogenesis, with lisinopril playing the role of antioxidant. Our preliminary results are encouraging, but further *in vivo* studies in different animal models are under progress in our laboratory for further elucidation of the role of ACE inhibitors in preventing cataract.

ACKNOWLEDGEMENT

The financial assistance of SRM University, SRM College of Pharmacy, Kattankulathur, Tamil Nadu, India is gratefully acknowledged.

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