Lakshmi and *Sharma*

LISINOPRIL FAILS TO PROTECT RAT LENS AGAINST SODIUM SELENITE INDUCED EXPERIMENTAL CATARACTOGENESIS

K.S Lakshmi[a], Shrinivas Sharma[b]*

**[a]Department of Pharmaceutical Analysis, SRM College of Pharmacy, SRM University, SRM Nagar, Kattankulathur- 603203. Tamilnadu, India.
[b]Department of Pharmacology, SRM College of Pharmacy, SRM University, SRM Nagar, Kattankulathur- 603203. Tamilnadu, India.**

Summary

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 lisinopril in preventing selenite-induced cataractogenesis. Nine days old Wistar rat pups divided into normal (Group I), control (Group II) and test (Group III) groups in which normal group injected normal saline s.c while control group injected with 19 μM/kg sodium selenite s.c. One day before the selenite challenge, the pups in the test group were injected intraperitoneally a single dose of 5 mg/kg of lisinopril and was repeated once daily for five consecutive days thereafter. At the end of the study period (30th postpartum day), slit-lamp examination of both eyes of each rat pup revealed definite nuclear cataract in both control and test group. The mean activities of the reduced glutathione, total protein and water soluble protein malondialdehyde (an indicator of lipid peroxidation), insoluble protein and lens Ca^{2+} concentration was non significant (P<0.05) in test group when compared with control group. These data suggest that lisinopril is unable to protect selenite-induced cataractogenesis.

*Corresponding author:
DR. Shrinivas Sharma
SRM College of Pharmacy,
SRM University,
Kattankulathur- 603203. Tamilnadu, India.
Tel.: +919962358436, fax.: 044 27455717
E-mail address: shrinivas.sharma29@gmail.com

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 (6-9). ACEi may act as ''magic bullets'' against oxidative stress and this may explain some of the beneficial effects of ACEi that cannot be associated to their action on blood pressure (10). Treatment with lisinopril or captopril showed to increase antioxidant enzymes and non-enzymatic antioxidant defenses in several mouse tissues (11). ACEi could also limit superoxide generation, and could modulate reactive oxygen and nitrogen species generation (12,13). ACEi can enhance the endogenous antioxidant defenses and suggest that this induction can protect cells from oxidant stress (11). The potential ability of ACEi to scavenge reactive oxygen and nitrogen species (RONS) has produced conflicting results (14,15). Some studies, mainly done in vitro, indicate that both, sulfhydryl-containing (i.e., captopril) and non-sulfhydryl-containing ACEi (i.e., lisinopril) can scavenge free radicals and attenuates selenite induced cataract in experimental setting (14). By contrast, other reports show that only sulfhydryl-containing ACEi are effective free radical/oxidant scavengers (16,17). In this report, we wanted to evaluate the in vivo clinical efficacy of lisinopril in sodium selenite induced cataract in rat pups.

Materials and Methods

Chemicals
Lisinopril was kindly provided by Torrent Pharmaceutical Ltd (Ahmedabad, Gujrat, India) approximate purity was 98%. All other chemicals and solvents were procured from SRL, Mumbai, India.

Treatment protocol
Nine day-old rat pups (Wistar strain) were used in this study. The pups were housed with parents in large spacious cages, and the parents were given food and water ad libitum. The animal room was well-ventilated and had a regular 12:12-h light/dark cycle throughout the experimental period. 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.

The rat pups were divided into three groups, each comprising 10 pups. In group I (normal), saline was injected intraperitoneally on postpartum day 10. In both experimental groups (II & III), sodium selenite (19 mmol/kg body weight) was injected subcutaneously on postpartum day 10. In addition, pups in group III received intraperitoneal injections (5 mg/kg body weight) of lisinopril; the first dose of lisinopril was administered 1 day prior to the selenite injection (that is, on postpartum day 9), and was repeated once daily for five consecutive days thereafter (on days 10 through 14). The dose of lisinopril was determined from previous study of Ghazi-Khansari et al., on liver mitochondrial cells culture study (18). The injections were given in the morning hours. On day 10 alone, Group III pups received lisinopril one hour prior to selenite injection.

Morphological assessment

The development of cataract in the rat eyes was assessed once a week for 3 weeks after selenite injection by slit-lamp biomicroscopy. Mydriasis was achieved by using a topical ophthalmic solution containing tropicamide with phenylephrine (Maxdil Plus, Hi-Care Pharma, Chennai, India). Only presence or absence of opacification of lens was documented without scoring the cataract by viewing under 12x magnification.

Following the final morphological examination at postpartum day 30, the animals were sacrificed by cervical dislocation; the lenses were at once dissected out for various biochemical studies. The paired lenses from each individual rat were pooled together and considered as one individual unit when estimating the various values.

Biochemical assessment

Reduced glutathione (GSH)

 The GSH content was estimated by the method of Moron et al (19). Two 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 (20). 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 (21) using bovine serum albumin as the standard.

Statistical analysis

All data were expressed as mean±SD. The groups were compared using one-way ANOVA with post-hoc Dunnett's test using group II as control and the chi-square test were applied wherever relevant. P<0.05 was considered significant.

Results and Discussion

Rat pups (Group II) treated with sodium selenite showed dense nuclear cataract in all 10 (100%) animals at the end of study. While, in lisinopril treated group (Group III) 8 out of 10 animals (75%) had dense nuclear cataract on 30th postpartum day. This difference was statistically insignificant (x^2 [df =1] = 2.28; P>0.05).

Group II animals showed significantly higher Na$^+$ (P<0.01) and Ca^{2+} (P<0.01) while lower K$^+$ (P<0.01) concentration compared with normal lenses. The values remained to be non significant (P<0.05) in lisinopril treated group also (Table 1).

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

Parameter	Group I	Group II	Group III
Sodium (Na^+) (%wt)	$0.173 \pm 0.001*$	0.219 ± 0.002	0.224 ± 0.004^{ns}
Potassium (K^+) (%wt)	$0.723 \pm 0.002*$	0.448 ± 0.002	0.425 ± 0.002^{ns}
Calcium (Ca^{2+}) (%wt)	$0.09 \pm 0.004*$	0.021 ± 0.002	0.020 ± 0.001^{ns}

All values are expressed as mean±SD of five determinations.
Group I: Normal, Group II: Selenite treated. Group III: Selenite and lisinopril.
Statistically different ($^{ns}P>0.05$, $^*P < 0.01$) when compared with group II values.

Selenite treated animals 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 not shown significant response in treated animals.

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

Parameter	Group I	Group II	Group III
Total Protein (mg/mg wt.)	$0.345 \pm 0.003*$	0.296 ± 0.012	0.311 ± 0.014^{ns}
Water Soluble Protein (mg/mg wt.)	$0.265 \pm 0.005*$	0.162 ± 0.001	0.174 ± 0.004^{ns}
Water Insoluble Protein (mg/mg wt.)	$0.072 \pm 0.002*$	0.156 ± 0.001	0.142 ± 0.002^{ns}

All values are expressed as mean±SD of five determinations.
Group I: Normal, Group II: Selenite treated. Group III: Selenite and lisinopril.
Statistically different ($^{ns}P>0.05$, $^*P < 0.01$) when compared with group II values.
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Moreover, 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 ($P<0.01$). 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 has not offered significantly protection (P>0.05) in test group because it neither increases the level of GSH nor decreases the lipid peroxidation (Table 3).

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

Parameter	Group I	Group II	Group III
GSH (μg/mg wt.)	2.45 ± 0.05*	1.16 ± 0.01	1.19 ± 0.01[ns]
MDA (μmol/g)	0.031 ± 0.001*	0.64 ± 0.02	0.57 ± 0.001[ns]

All values are expressed as mean±SD of five determinations.
Group I: Normal, Group II: Selenite treated. Group III: Selenite and lisinopril.
Statistically different ([ns]P>0.05, *P < 0.01) when compared with group II values.
GSH:glutathione; MDA:malondialdehyde.

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 (5,22).

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 (23). 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 (24). This study showed lower total and water-soluble proteins and K^+ ions whereas higher water insoluble protein and Na^+ as well as Ca^{2+} ions concentration with selenite as well as lisinopril treated group. There were no significant alteration was observed due to lisinopril treatment.

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 (25). Restoration of GSH and MDA levels, protection against aggregation and insolubilization of lens proteins, and maintenance of lens clarity was documented in our previous study in which we have evaluated the anticataract activity of captopril (26).

These findings and the results of the past study therefore show that among the thiol and nonthiol ACEi, captopril was able to quench ROS generation from rat lenses (26). Captopril, the first described, is a thiol compound which can react with superoxide anion radical acting as a scavenger, or with hydroxyl radical (27-31). Considering the chemical structure of most of the ACEi used, the latter assessment seems to be the correct one, since non-sulfhydryl-containing ACEi molecules lack chemical groups that can scavenge radicals.

In conclusion, lisinopril fails to prevent sodium selenite induced cataract because it fails to prevent the quenching of free radical generation. We can say that, this difference is important to check the clinical use of lisinopril over captopril or other thiol containing ACE inhibitors. Our preliminary results are encouraging, but further *in vivo* studies in different animal models are under progress in our laboratory for further differentiation of ACE inhibitors based on their anticataract potential.

Acknowledgements

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