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Allylmercaptocaptopril attenuates sodium selenite induced experimental cataractogenesis: an in vitro and in vivo study

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Abstract:

The present study sought to assess the efficacy of the allylmercaptocaptopril in preventing selenite-induced cataractogenesis in an experimental setting. The first, in vitro phase of the study was performed on lenses from Wistar rats incubated for 24 h at 37°C 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 5 mM allylmercaptocaptopril was added (Group III). The second, in vivo phase of the study, 9 days old Wistar rat pups divided into normal, control and test groups were injected subcutaneously with 19 μM/kg sodium selenite except normal group. One day before the selenite challenge, the pups in the test group were injected intraperitoneally a single dose of 50 mg/kg of allylmercaptocaptopril and was repeated once daily for five consecutive days thereafter. When the pups first opened their eyes (when the pups were 15 days old), their eyes were examined by slit-lamp biomicroscopy. In the first phase, the mean activities of reduced glutathione, total protein, water soluble protein, Ca2+ ATPase activity and the antioxidant enzymes activity were significantly lower in Group II than in Group I or Group III lenses, while malondialdehyde concentration (an indicator of lipid peroxidation) and lens calcium ion concentration was significantly higher in Group II lenses than that in Group I or Group III lenses. The second, in vivo phase of the study revealed dense opacification (cataract formation) in 100% of Wistar rat pups receiving sodium selenite alone (19 μM/kg s.c) but in only 12.5% of those receiving subcutaneous selenite and allylmercaptocaptopril (50 mg/kg i.p). The study on the evaluation of the antacataract potential of allylmercaptocaptopril in experimental animals indicated that it modulates antioxidant parameters in the enucleated eye lenses. It also attenuates selenite-induced cataract both in vitro and in vivo, so it may be useful for cataract therapy.

Keywords: Allylmercaptocaptopril; Oxidative stress; Cataractogenesis; Sodium selenite
Introduction

Cataract formation is one of the leading causes of blindness throughout the world. Forty-two percent of visual blindness is attributed to cataract [1]. Currently, 17 million people around the world are blind due to cataract [2], among which, one half of the cases are from developing countries of Asia and Africa [3]. Apart from higher prevalence, onset of cataract is at least 20 years earlier in developing countries as compared to the developed world. Cataract is a multifactorial disease associated with several risk factors such as aging, diabetes, malnutrition, diarrhea, sunlight, smoking, hypertension and renal failure [4]. Free radical-induced oxidative stress is postulated to be perhaps the major factor leading to senile cataract formation [5]. Oxidative stress originates due to an imbalance between the generation of reactive oxygen species (ROS) and their removal. Abnormally high levels of free radicals due to an over production/inadequate removal and simultaneous decline in antioxidant defence mechanism can damage cellular proteins, nucleic acids and membrane lipids [5], Reactive oxygen species also initiate lipid peroxidation of polyunsaturated fatty acids (PUFAs) resulting in the production of reactive carbonyl compounds, among which malondialdehyde (MDA) is most abundant. Physiologic antioxidants such as pyruvate and nutritional antioxidants such as ascorbate, vitamin E, and carotenoids were found to delay the development of experimental cataract [5, 7],

Several previous experimental findings postulated that angiotensin converting enzyme inhibitors (ACEI) may act as a “Magic bullets” against oxidant stress especially captopril which exhibits a wide variety of biological activities [8-11]. In our previous study, we have determined the anticitrataractogenic effect of captopril against selenite induced cataract in experimental animals [12].

Recently, antioxidant properties of garlic were also suggested by showing that organosulfur compounds from garlic inhibited the peroxidation of lipids and possesses anti oxidant and radical scavenging activity [13, 14]. These health related properties of garlic are attributed to the organosulfur compounds, particularly to allicin, the pungent smelling compound. Allicin is produced during the crushing of garlic by the interaction of alliin, the non-protein amino acid, with the pyridoxal phosphate containing enzyme, allinase.

Unfortunately, allicin is only produced by freshly crushed garlic and is temperature sensitive, and its activity decays with time. Allicin can covalently react with captopril to form allylmercaptocaptopril (AMC), in which the allyl moiety is linked via a disulfide bond to captopril [15, 16]. Studies in Sprague-Dawley rats fed with a high-fructose diet suggest that allylmercaptocaptopril combined some of the therapeutic actions of captopril with those of the natural product allicin on blood pressure, measures of glucose and lipid metabolism, and markers of renal function in the SHROB model of metabolic syndrome [17]. In view of the antioxidant properties of captopril and allicin, and since oxidative stress has been implicated in cataractogenesis, we reasoned that allylmercapto­captopril might exhibit anticataractogenic potential. This hypothesis evaluated an in vitro and in vivo (in an experimental animal model) potential of allylmercap­to­captopril in selenite induced cataract.

Materials and Methods

Chemicals

Captopril was kindly provided by Wockhardt Ltd (Aurangabad, Maharashtra, India) approximate purity was 98%. Dulbecco’s modified Eagles medium (DMEM) was obtained from Hi Media Laboratories (Mumbai, India). Fetal bovine serum (FBS), sodium selenite, diallyl disulfide and chemicals required for enzyme assay were purchased from Sigma Chemical Company, St. Louis, MO. Twenty-four-wells Falcon plastic culture plate was acquired from Genei, Bangalore, India. All other chemicals and AR grade solvents were procured from SRL, Mumbai, India.

Synthesis of allylmercaptocaptopril

Allylmercaptocaptopril (AMC) was synthesized by previous reported method by Miron et al [16], with some major modifications. First, Allicin (diallyl disulfide oxide, Fig. 1) was synthesized by a modified method of Stoll and Seebeck [18]. Allylmercaptocaptopril (AMC) was synthesized by the addition of captopril solution (1 mM) in 7.5 ml water, pH 5.5) to an aqueous solution of 0.55
mmol allicin. The reaction was monitored by HPLC analysis until captopril was no longer detected. The reaction mixture was then acidified by hydrochloric acid and extracted by ether to remove non-reacted allicin. The water phase was extracted with ethyl acetate. The organic phase was dried by Na₂SO₄, filtered and evaporated to remove water. The organic phase was then re-dissolved in ethanol and dried by a speed vacuum concentrator. The reaction product was analyzed by HPLC and its structure confirmed by IR, NMR and mass spectrometry. IR (KBr) spectra were recorded on a Perkin Elmer FTIR spectrometer (vmax in cm⁻¹) and ¹H NMR spectra were recorded in CDCl₃ on a Bruker 500 MHz Avance spectrometer using TMS as internal reference (Chemical shift in ppm). Mass spectra were recorded using Agilent HP5937 spectrometer. The purity of the synthesized compound was checked by HPLC. The HPLC system consisted of a Shimadzu Class LC-10AT vp and LC-20AD pumps connected with SPD-10A vp UV-visible detector. The data acquisition was performed by Spincotech 1.7 software. The system was equipped with reverse phase column Gemini C₁₈ (150 mm × 4.6 mm i.d., 5 µm) (Phenomenex, Torrance, USA). The mobile phase consisted of 60% methanol and water containing 0.1% formic acid at a flow rate of 0.8 mL/min. The retention time was found to be 11.6 min. Spectral data (¹H NMR, IR and mass) of the synthesized compound was in full agreement with the proposed structure. IR (KBr) ν cm⁻¹: 3459 (OH), 2984 (C-H), 1728 (C = O), 939 (ν). ¹H NMR (DMSO) δ (ppm): 10.02 (s, 1 H, OH), 5.02 (t, 2H, SCH₂), 1.24 (q, 3H, CH₃). MS: m/z 289 (M⁺). The reaction between captopril and allicin to form allylmercaptocaptopril is illustrated in Fig. 1.

In vitro study

Wistar rats of either sex in the weight range 80 to 100 gm 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 (which contained sodium bicarbonate (0.2% w/v) but did not contain calcium), supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, 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 100 µM. Lenses were maintained in a 24-well culture plate with 2 ml medium and lenses were incubated for 24 h under 5% CO₂ at 37°C in a CO₂ 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 5 mM AMC. The dose of AMC was taken as equimolar dose of captopril according to our previous study [12]. All lenses were incubated for 24 h at the conditions described earlier. After incubation, lenses were processed for photographic evaluation and estimation of biochemical parameters.

Figure 1 Schematic illustration of the chemical reaction between captopril and allicin that forms allylmercaptocaptopril
Reduced glutathione (GSH)

The GSH content was estimated by the method of Moron et al. [19]. 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 NaH2PO4 and 0.5 ml of 0.6 mM 5,5'-dithiobis-2-nitrobenzoic acid in 1% trisodium citrate was added in successition. The intensity of the resulting yellow color was read spectrophotometrically at 410 nm. Reduced GSH was used as a standard.

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 successition. 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 MDA formed in µmol/g wet weight for lenses.

Assay of Ca2+ ATPase activity

The activity of Ca2+ ATPase in the lens samples was measured by the method of Ronive and Kienzleler [21]. To the reaction tube, 0.25 ml of substrate (40 mM ATP in 0.4 M Tris-HCl buffer, pH 7.4) and 0.1 ml of lens homogenate was added. A tube devoid of the homogenate served as a control. All the tubes were incubated for 30 min in a water bath at 37°C. The incubation was stopped by adding 2 ml of 10% trichloroacetic acid (TCA) then 0.2 ml ATP were added to control tubes and these tubes were subsequently kept in ice for 20 min. All the tubes were then centrifuged at 2500 x g for 10 min and the supernatant was collected. The protein-free supernatant was analyzed for inorganic phosphate. For this, 3 ml of the supernatant were treated with 1 ml of ammonium molybdate and 0.4 ml of 2.4 aminonapthol sulphonic acid (ANSAS). The color developed was read at 680 nm after 20 min.

Estimation of levels of Ca2+

The levels of calcium ions in the lenses were estimated as follows. Individual lenses were weighed and digested in concentrated nitric acid : perchloric acid (5 : 1). After complete digestion, the samples were dried, diluted with 1% nitric acid, and made up to 50 ml in a standard flask. The samples were analyzed by flame photometry and the results were expressed as µM/mg protein of lens. Calcium carbonate was used as a standard that was prepared by dissolving in 1 per cent nitric acid.

Estimation of protein values

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 fractions of the protein were estimated by preparing homogenate in double distilled water. The water-soluble supernatant was used for estimation of soluble protein. The protein content of the samples was determined by the method of Lowry et al. [22] using bovine serum albumin as the standard.

Enzyme assays

A separate set of experiments was conducted under the same conditions as described above. After 24 h of incubation, 10% (w/v) homogenate of lenses from each group was prepared in 50 mM phosphate buffer (pH 7.0). The enzyme activities such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH) and glutathione-S-transferase (GST) were measured in the supernatant obtained by the centrifugation of the homogenate at 5000 rpm for 15 min at 4°C. Monitoring spectrophotometrically at 480 nm, the ability of the enzyme to inhibit the oxidation of epinephrine [23] was
used to assess the activity of superoxide dismutase (SOD). One unit of SOD activity was defined as the amount of enzyme inhibiting 50% of the rate of autoxidation of epinephrine under the defined assay conditions. The enzyme activity of catalase (CAT) was measured spectrophotometrically at 240 nm by following the decomposition of \( \text{H}_2\text{O}_2 \) [24]. One unit of CAT activity represented the amount of enzyme required to decompose 1 \( \mu \) mole of \( \text{H}_2\text{O}_2 \)/min. Glutathione peroxidase (GPx) activity was monitored at 340 nm [25]. One unit of enzyme activity was defined as the amount of GPx required to use 1 \( \mu \) mole of nicotinamide adenine dinucleotide phosphate per minute. The conjugation of GSH with 1-chloro, 2,4-dinitrobenzene (CDNB), a hydrophilic substrate, was examined spectrophotometrically at 340 nm [26] to measure glutathione S-transferase (GST) activity. One unit of GST was defined as the amount of enzyme required to conjugate 1 \( \mu \) mole of CDNB with GSH per minute.

**In vivo study**

Cataract was induced in 9-d-old Wistar rat pups. The normal, control and test groups had equal numbers of pups. Eight pups in each group were injected subcutaneously with 19 \( \mu \)M/kg body weight of sodium selenite except normal group which was fed with normal saline. One day before the selenite challenge, the pups in the test group were injected intraperitoneally a single dose of 50 mg/kg of allylmercaptocaptopril and was repeated once daily for five consecutive days thereafter. When the pups first opened their eyes (when the pups were 15 days old), their eyes were examined by slit-lamp biomicroscopy and were also photographed (Topcon Digital Camera Unit DC-1, Japan). Cataracts were observed in both groups on postnatal day 16, when the eyes of the pups first opened. Mydriasis was achieved by using a topical ophthalmic solution containing tropicamide with phenylephrine (Maxdil Plus, Hi-Care Pharma, Chennai, India).

**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 100 \( \mu \)M group as control and the chi-square test was applied wherever relevant.

**Results**

**In vitro study**

As shown in Fig. 2, after 24 h of incubation in selenite 100 \( \mu \)M, lens becomes completely opaque after 24 h of incubation in selenite [c & d] (complete loss of transparency, no grid lines visible through lens) and after 24 h of incubation in selenite + AMC lens appears slightly hazy [e & f] (grid lines are visible).
(c & d) as against lenses incubated in DMEM alone (a & b). Incubation of lenses with AMC 5 mM seem to retard the progression of lens opacification compared with control group (selenite 100 μM). This is because more number of grid lines are clearly visible in AMC supplemented lenses (e & f) than in selenite-treated lenses.

The mean GSH value in the normal lenses was 2.98 ± 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 AMC, 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 2.28 ± 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 (1.147 ± 0.02 pmol/g of fresh weight of lens, P < 0.01). AMC supplementation significantly protected (P < 0.01) the test group lenses from lipid peroxidation: the MDA content was 0.045 ± 0.001 pmol/g of wet weight of lens (Table 1).

Selenite 100 μM treated lenses also showed significantly low concentrations of proteins (total and water soluble proteins) in the lens homogenate (P < 0.01) and very high Ca²⁺ concentration (P < 0.01) compared with normal group having normal lenses (Table 2). Captopril group had significantly higher concentrations of total lens proteins and water soluble protein (P < 0.01), compared with selenite 100 μM group. At the same time, they had lower Ca²⁺ conc. (P < 0.01) compared with selenite 100 μM group. Activity of the membrane ionic pump, Ca²⁺ ATPase, was found to be decreased significantly following selenite induction whereas, treatment with AMC was found to maintain activity close to the normal levels (Fig. 3).

The effect of 5 mM AMC on different enzymes (SOD, CAT, GPx and GST) is presented in Table 3. It was observed that in presence of selenite stress in group II lenses, antioxidant enzymes were significantly reduced as compared with the normal group. In presence of captopril, there was a significant positive modulation of enzyme activities observed in group III lenses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (μg/mg wt.)</td>
<td>2.98 ± 0.05*</td>
<td>1.36 ± 0.01</td>
<td>2.28 ± 0.01*</td>
</tr>
<tr>
<td>MDA (μmol/g)</td>
<td>0.061 ± 0.001*</td>
<td>1.157 ± 0.020</td>
<td>0.045 ± 0.001*</td>
</tr>
</tbody>
</table>

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 AMC. Statistically significant difference (*P < 0.01) when compared with group II values. GSH: glutathione; MDA: malondialdehyde.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (mg/mg wt.)</td>
<td>0.421 ± 0.003*</td>
<td>0.319 ± 0.012</td>
<td>0.368 ± 0.014*</td>
</tr>
<tr>
<td>Water soluble protein (mg/mg wt.)</td>
<td>0.312 ± 0.005*</td>
<td>0.192 ± 0.001</td>
<td>0.248 ± 0.004*</td>
</tr>
<tr>
<td>Calcium (Ca²⁺) (μg/mg protein)</td>
<td>1.36 ± 0.17*</td>
<td>3.79 ± 0.12</td>
<td>1.94 ± 0.13*</td>
</tr>
</tbody>
</table>

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 AMC. Statistically significant difference (*P < 0.01) when compared with group II values.
Figure 3 Activity of Ca$^{2+}$ ATPase in lens. 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 AMC. Statistically significant difference (*p < 0.01) when compared with group II values.

Table 3 Levels of antioxidant enzymes in Group I, Group II and Group III lenses.

<table>
<thead>
<tr>
<th>Parameter (U/mg protein)</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD 2.46 ± 0.14*</td>
<td>0.26 ± 0.03</td>
<td>1.92 ± 0.01*</td>
<td></td>
</tr>
<tr>
<td>CAT 1.19 ± 0.001*</td>
<td>0.01 ± 0.01</td>
<td>0.89 ± 0.02*</td>
<td></td>
</tr>
<tr>
<td>GPx 10.73 ± 0.91*</td>
<td>1.07 ± 0.10</td>
<td>8.62 ± 0.17*</td>
<td></td>
</tr>
<tr>
<td>GST 2.06 ± 0.19*</td>
<td>0.16 ± 0.01</td>
<td>1.28 ± 0.13*</td>
<td></td>
</tr>
</tbody>
</table>

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

Figure 4 Topcon photo-slit-lamp images of rat eyes on 16th day after injection. Clear lens in normal group (A), nuclear cataract in sodium selenite group (B) and clear lens in sodium selenite + AMC treated group (C).
In vivo study

As shown in Fig. 4, rat pups that had received selenite alone (B), dense opacification of the lens was observed in all (100%) 8 animals. In contrast, only 1 of 8 (12.5%) rat pup in test (received selenite and AMC) exhibited slight vacuolization but not opacification otherwise others were clear (C) in which Group A animals served as a normal (A). This difference was statistically significant ($\chi^2$ [df = 1] = 9.6; $P < 0.01$).

Discussion

Garlic (*Allium sativum*) has been used as a treatment for several diseases for thousands of years [27]. Allicin is one of the most active constituents of garlic extract, also responsible for its characteristic odor. Anti-oxidation and free radicals trapping are part of the mechanisms underlying its wide spectrum of pharmacological activities [28] which includes lowering of blood pressure and insulin [29], improvement of lipid profile [30], anti-atherosclerotic and anti-coagulant activity [31,32].

Oxidative stress has been suggested as a common underlying mechanism of cataractogenesis, and augmentation of the antioxidant defenses of the lens has been shown to prevent or delay cataract [33]. Different agents with diverse chemical structures have shown antioxidant properties in different systems, and their beneficial effects have been demonstrated in various pathologic conditions including cataract. Based on our previous findings on captopril as anticataractogenic agent [12], we predicted that allylmercaptocaptopril, a covalent conjugate of allicin with the ACE inhibitor captopril, would have a greater impact and therapeutic efficacy in correcting oxidative stress induced cataract in animals than captopril alone.

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. A single dose of selenium administration leads to impaired oxidative defense, membrane damage and cataract formation [34]. The lens in vitro is highly susceptible to damage by reactive oxygen species (ROS), as evidenced by loss of transparency and decreased active transport of cations, GSH and ATP, as well as protein insolubilization and generation of lipid peroxides [35, 36].

In the present study, biochemical 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 and decrease water soluble protein content. Such changes in GSH and MDA levels in presence of selenite have been reported [37]. 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 AMC.

Calcium is essential for various lens fiber cell processes including its differentiation [38]. The levels of the divalent cation Ca$^{2+}$ in the lens are maintained at submicromolar range and are lower than that of the aqueous humor [39]. It has also been found that alterations in the homeostasis of lenticular Ca$^{2+}$ have been implicated in cataractogenesis [40]. In this study, activity of Ca$^{2+}$-ATPase was significantly decreased in selenite treated lenses with a corresponding increase in Ca$^{2+}$ concentration. Treatment with AMC was observed to maintain the activity of Ca$^{2+}$-ATPase and level of Ca$^{2+}$ close to normal range. The level of Ca$^{2+}$ is maintained by Ca$^{2+}$-ATPase, which counteracts the inward passive diffusion of Ca$^{2+}$ [41]. Ca$^{2+}$-ATPase is a major factor involved in maintaining lenticular Ca$^{2+}$ levels and loss of its activity could explain the rise in Ca$^{2+}$. Oxidation of the critical sulfhydryl groups of Ca$^{2+}$-ATPase on lens epithelial membrane, influx of calcium from the aqueous humor, activation of calpain, cleavage of N-terminal extensions of β-crystallins of the lens, interaction between exposed charged groups, and the formation of insoluble protein aggregates are some of the steps leading to the development of opacification. Selenite-induced opacification of the lens in vitro also has been demonstrated [42, 43]. These findings are in agreement with our observation that selenite-induced oxidative stress resulted in higher levels of lipid peroxidation, loss of
activity of Ca\(^{2+}\)-ATPase, and accumulation of Ca\(^{2+}\) in the lens. The lower levels of Ca\(^{2+}\), higher activity of Ca\(^{2+}\)-ATPase, and decreased levels of lipid peroxidation in the lens of the allylmercaptocaptopril-treated group could be attributed to its antioxidant protection against selenite-induced oxidative stress.

SOD, CAT, GPx and GST are important components of the innate enzymatic defenses of the lens. SOD, a chain-breaking antioxidant, was first described by McCord and Fridovich [44] in red blood cells. Varma et al. [45] first described its occurrence in the lenses of different species. SOD converts superoxide to \(\text{H}_2\text{O}_2\). The enzyme exists in two forms, one containing Mn\(^{2+}\), restricted to the mitochondria, and a cytosolic form containing Zn\(^{2+}\) and Cu\(^{2+}\). The occurrence of GPx in the lens was first shown by Pirie [46]. GPx is required to check lipid peroxidation initiated by superoxide in the phospholipid bilayer, for maintenance of membrane integrity. CAT is a hemoprotein that requires NADPH for regeneration to its active form [47]. The presence of CAT in the lens has been well demonstrated [48]. Both CAT and GPx catalyse the transformation of \(\text{H}_2\text{O}_2\) within the cell to harmless by-products, thereby curbing the quantity of cellular destruction inflicted by products of lipid peroxidation [49]. GST is important for detoxification process. The level of these enzymes were significantly diminished with selenite and positively modulated in the presence of AMC. The data clearly demonstrated that AMC significantly improves the antioxidant defense mechanisms of the normal lens.

Based on our findings of in vitro studies that AMC acts as an antioxidant, it was evaluated against selenite-induced cataracts in young rats. AMC significantly protected the lens morphology and clarity: 12.5% of the eyes had slight vacuolization; in contrast, 100% of the control eyes developed dense opacity or nuclear cataract. From the current study, it is evident that AMC protects the lens against oxidative stress. Our results on selenite-induced cataracts in vitro and in vivo not only demonstrate the protective effect of AMC but also indicate that it prevents cataractogenesis by virtue of its antioxidant properties. Allylmercaptocaptopril, therefore, may be useful for prophylaxis or therapy against cataracts.

Conclusion

In conclusion, the study on the evaluation of the anticitaract potential of allylmercaptocaptopril in experimental animals indicated that it modulates antioxidant parameters in the enucleated eye lenses. It also attenuates selenite-induced cataract both in vitro and in vivo, so it may be useful for cataract therapy.

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