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ORIGINAL ARTICLE

Prevention of Selenite-induced Cataract by L-Cysteine and Vitamin C in Rats

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Abstract

Background: Development of a drug which could prevent or delay the onset or progression of cataract will help to reduce the number of people getting blind due to cataract worldwide. This study was undertaken to evaluate the clinical and biochemical changes of the crystalline lens and gel-electrophoresis of water soluble proteins in a selenite-induced cataract and to assess the preventive role of L-Cysteine and vitamin C in rat as an animal model.

Methods: Cataracts were induced in rats by administration of sodium selenite. In control group, saline was injected subcutaneously (SC). In experimental groups (groups 2-5), sodium selenite (20 µmol/kg) was injected SC. Rats in group 3 received SC injections of 0.1 ml of vitamin C (0.3 mM), in group 4 received SC injection of 0.1 ml of L-cysteine (0.05 µmol) and those in group 5 received SC injection of 0.1 ml of L-cysteine (0.1 µmol). The development of cataract was assessed clinically. Then, the lenses were checked for total and soluble protein concentrations and eletrophoretic pattern (SDS-PAGE).

Results: Sodium selenite could induce cataract and cause biochemical and eletrophoretic changes in the lens. L-cysteine and vitamin C were highly effective in preventing or minimizing selenite-induced cataract and in maintaining near-normal total protein and soluble protein concentrations of the lens. These reagents were also effective in restoring the near normal pattern of lens proteins in SDS-PAGE. L-cystein was more effective than vitamin C in prevention of cataract but the difference was not statistically significant.

Conclusions: Our results showed that cataractous and biochemical changes of the crystalline lens proteins due to selenite can be minimized or prevented by L-cysteine and vitamin C.

Keywords: Cataract; L-Cysteine; Vitamin C; Selenite; Rat

Introduction

Cataract is the most common cause of blindness worldwide and its incidence will increase as the world's population ages.[1] Looking for preventive methods to delay the onset of cataract can help to narrow the gap between the incidence of cataract blindness and the provision of surgical treatment.

Even in modern ophthalmology, there is no effective medical treatment for cataract except surgery. Although effective, surgical remedy has its own limitations. Development of a drug which could prevent or delay the onset of cataract will lessen this burden and reduce the number of blind patients waiting for cataract surgery. It is widely accepted that oxidative stress is a significant factor in the pathogenesis of cataract both in experimental animal models[2,3] and in cultured lens systems.[4-6] According to oxidative hypothesis of cataract formation, reactive oxygen species can damage lens proteins and lens fiber cell membrane. Therefore, nutrients with antioxidant capabilities can potentially protect against these changes.[7-9]

A significant number of epidemiological studies have been published regarding the potential role of antioxidants in the prevention of cataract.[10-18] Although the majority of these studies have shown a positive correlation between higher dietary antioxidant intake and decreased cataract formation, [10-14]

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conflicting results exist between different epidemiological studies.[15-18]

In the present study, we have investigated the role of L-Cysteine and vitamin C in preventing cataract and also we evaluated changes in protein and electrophoretic pattern of the lens water, soluble proteins in a selenite cataract model in rats and assessed the role of L-Cysteine and vitamin C in the prevention of these changes.

Materials and Methods

Sodium selenite (Merck, Darmstadt, Germany), bovine serum albumin, chemicals for SDS-PAGE, and buffer salts (Sigma Chemical Co., St Louis, MO, USA), L-cystein hydrochloride (BDH Chemical Company, England), and sodium ascorbate (FLUKA Company, Switzerland) were prepared. All other chemicals and solvents were of analytical grade. Rats of Sprague-Dawley strain were provided by the Laboratory Animal Research Center of Shiraz University of Medical Sciences. Animal handling, maintenance and experimentations were done in accordance with the guidelines set by the Institutional Animal Ethical Committee. The animal room was well-ventilated and had a regular 12:12-h light/dark cycle throughout the experimental period. The rats were divided into one control and four experimental groups. Group 1 (control) consisted of 40 rats (1a: 16 rats and 1b: 24 rats), group 2, 40 rats (2a: 16 rats and 2b: 24 rats), group 3, , 40 rats (3a: 18 rats and 3b: 22 rats), group 4, 32 rats (4a: 14 rats and 2b: 18 rats) and group 5 consisted of 20 rats (5a: 10 rats and 5b: 10 rats). In group 1, 100 μg of saline was injected subcutaneously on postpartum day 10. In the four experimental groups (2-5), 100 μl of sodium selenite solution (20 μmol/kg of dissolved solution in distilled water) was injected subcutaneously on postpartum day 10. In addition, rats in group 3 received subcutaneous injections of 0.1 ml of vitamin C (0.3 mM) on postpartum day 8 (2 days prior to the selenite injection) and this injection was repeated once daily for 14 and 28 consecutive days thereafter in groups 3a and 3b, respectively. Rats in group 4 received subcutaneous injections of 0.1 ml of L-cysteine (0.05 μmol) on postpartum day 8 (2 day prior to the selenite injection) and the injection was repeated every other day for 14 and 28 consecutive days thereafter in groups 4a and 4b, respectively. Rats in group 5 received subcutaneous injections of 0.1 ml of L-cysteine (0.1 μmol) on postpartum day 8 (2 days prior to the selenite injection) and the injection was repeated every other day for14 and 28 consecutive days thereafter in groups 5a and 5b, respectively.

Development of cataract in the rats' eyes was assessed every other day for 2 weeks after selenite injection using slit-lamp biomicroscopy by an ophthalmologist. Prior to each examination, mydriasis was achieved in each eye by instilling one drop of a 0.5% tropicamide every 10 min for 3 times. The eyes were viewed under a slit-lamp biomicroscope at 16× magnification; the observer was blind to the group of the animals before classifying the cataracts. Any cataracts developed were classified as below and photographed. Lens opacification was classified into no opacification (clear lens), subcapsular cataract, nuclear cataract and mature cataract (lens with a dense opacity involving the entire lens).

Following the final examinations, the animals in subgroups 1a, 2a, 3a, 4a, 5a were sacrificed by high dose inhalation of ether on day 14 after administration of selenite while the subgroups 1b, 2b, 3b, 4b, 5b were euthanized on the 28th day. Enucleation was done and the lenses were dissected out for various biochemical and eletrophoretic studies.

The lenses from each group of rats were homogenized in 10 mM phosphate buffer (pH=8), and centrifuged at 3500 rpm for 3 min at 4°C. The supernatant obtained was stored at −20°C; being used for the analysis of total and soluble protein concentration, and SDS-PAGE. The total protein content of the samples was determined by the method of Lowry et al.[19] using bovine serum albumin as the standard. Soluble protein concentration was measured spectrophotometrically. The mentioned supernatants were diluted with distilled water to achieve 1/20 dilution and the absorbences were recorded at 280 and 260 nanometer and then protein concentration was measured using the following formula: mg protein/ml=(A280X1.55)-(A260X0.76).

SDS slab polyacrylamide gel electrophoresis was conducted according to the Laemmli's method (1970); using 12.5% acrylamide separating gel.[20] Protein samples were added to the loading buffer to give a final concentration of 1 mg/ml protein, 0.01 mol/ml Tris-HCl, pH=6.8, 0.4% SDS, 100 g/l glycerol and 0.04 g/l bromophenol blue. The running gel with dimensions of 140×140×1 mm was made of 50-200 g/l gradient polyacrylamide in 1.2 mol/l Tris-HCl , pH=8.8 and 3 g/l SDS. The stacking gel contained 30 g/l acrylamide in 0.25 mol/l Tris-HCl, pH=6.8 and 2 g/l SDS. The electrode buffer was comprised of 0.025

mol/l Tris-HCl, 0.192 mol/l glycine and 0.15 % SDS at pH=8.16. Electrophoresis was performed at a constant 25 mA current and the gel was stained with 0.25% Coommassie Brillint Blue R-250 in 50% acetic acid/25% methanol and de-stained with 10% acetic acid/ 7% methanol.

All statistical calculations were carried out with the SPSS software (version 11.5, Chicago, IL, USA). The values are expressed as the mean±SD. The data were statistically analyzed; using Chi-Square test to evaluate differences in cataract pattern among all 5 groups, one way ANOVA (comparison of total and soluble proteins among 5 groups) and two way ANOVA (comparison of the effect of days of sampling, i.e. 14 and 28). There was a significant difference between means; using Duncan's multiple range test at the level of $p<0.05$.

Results

Table 1 shows the effect of different reagents on cataract formation in rats of 5 groups. On postpartum day 24th (2 weeks after selenite injection), the rats were evaluated for cataract development. None of the rats in the control group (group 1, no selenite) had cataract. In group 2, all of the rats (100%) had developed cataracts classified as subcapsular, nuclear and mature cataract (lens with a complete dense opacity involving the entire lens). However, in group 3, which had received vitamin C injections in addition to selenite, only 20 of 40 (50%) rats developed cataract (mature cataract in 12 rats and nuclear cataract in 8 rats). This difference (between groups 3 and 2) was statistically significant (P=0.001). The remaining 20 of 40 (50%) rats of group 3 had clear, normal lenses.
In group 4 which received subcutaneous injections of 0.1 ml of L-cysteine and selenite, 24 rats out of 32 (75%) had clear normal lenses and only 8 (25%) of them developed cataract (mature cataract in 6 rats, posterior subcapsular in 1 and nuclear in 1). This difference in cataract formation (groups 2 and 4) was statistically significant (P=0.001).

In group 5, which received subcutaneous injections of 0.1 ml of L-cysteine, 16 rats out of 20 (80%) had clear normal lenses at the examination on postpartum day 24th and only 4 (20%) rats developed cataract (mature cataract in 3 rats and nuclear in 1 rat). This difference (between groups 2 and 5) was statistically significant (P=0.001). The comparison of the two concentrations of L-cystein and vitamin C showed that L-cystein was more effective than vitamin C in prevention of cataract (P=0.030 for comparison of group 3 with group 4 and P=0.020 for comparison of group 3 with group 5). The comparison of the two concentrations of L-cystein showed no statistically significant difference in the prevention of cataract. (P=0.740). A significant difference was observed between day 14 and day 28 post-selenite injection.

Table 2 shows the effects of different treatments on the proteins of the lens. After 14 and 28 days post-selenie injection, the total lens protein concentration (expressed as mg protein/ml lens extract) was significantly (P=0.050) lower than those in group 2 (selenite alone) as compared with group 1 (control), group 3 ((selenite+vitamin C) and groups 4 and 5 (selenite+L-cysteine). After 14 days, the mean concentrations of soluble protein in the lenses of group 2a rats were

Table 1: The effect of different reagents on cataract formation in rats.

Groups	Intervention	No. of eyes	Examination at 24 days old
Group1	Control(saline)	40	Clear lens
Group2	Selenite sodium (20 µmol/kg)	40	28 mature cataract 6 nuclear cataract 6 posterior subcapsular cataract (PSCC)
Group3	Selenite and vitamin C (0.1 ml of 0.3 mM)	40	12 mature cataract 8 nuclear cataract 20 clear lens
Group4	Selenite and L-cysteine (0.1 ml ,0.05 µmol)	32	6 mature cataract 1 nuclear cataract 1 PSC C 24 clear lens
Group5	Selenite and L-cysteine (0.1 ml ,0.1 µmol)	20	3 mature cataract 1 nuclear cataract 16 clear lens

Table 2: Mean and ±SD of total and soluble protein content of lenses of rats treated by different reagents.

Groups	Days after selenite injection	No. of eyes	Total protein (mg/ml)	Soluble protein (mg/ml)
1a	14	16	439.0±278.0	423.5±65.5
1b	28	24	886.0±193.5	635.8±215.6
2a*	14	16	354.0±145.0	193.5±89.1
2b**	28	24	156.0±68.0	265.6±152.1
3a	14	18	704.5±226.0	382.3±78.2
3b	28	22	508.0±211.0	189.4±114.9
4a	14	14	432.0±99.0	434.0±151.9
4b	28	18	516.0±197.0	187.7±97.4
5a	14	10	582.0±308.0	311.0±78.0
5b	28	10	982.0±422.0	379.2±36.8

*****Values of group 2a were significantly different from those of group 1a, 3a, 4a and 5a (P<0.050).
******Values of group 2b were significantly different from those of group 1b and 5b (P<0.050).

significantly (*P*=0.050) lower than those of groups1a, 3a, 4a and 5a rats. After 28 days, the mean concentrations of soluble protein in the lenses of group 2b rats were significantly (*P*=0.050) lower than those of group 1b and 5b rats but not significantly different from groups 3b and 4b rats (P>0.050).

The SDS-PAGE profile of the lens proteins was carried out to visualize the effect of sodium selenite and prophylaxis with viamin C and L-cysteine. Group 1 (a and b) showed the normal protein profile. In group 2 (a and b), two bands were detected which had a lower intensity than that of the control group and corresponded to proteins with 18 kDa to32 kDa and more than 45 kDa molecular weights. These bands showed near to normal expression in groups 3 (a and b), 4 (a and b) and 5 (a and b), as found in group1.

Discussion

Cataract presents by opacities of the lens and is closely associated with aging and with several metabolic diseases. Cataract remains the major cause of curable blindness, accounting for more than half of the total blindness.[1] Causative factors of cataract are multiple and the etiology is unclear, but oxidative stress is thought to be one of the major underlying factors in most cataracts.[21] Various experimental models have been used to study the etiology of cataract and to investigate different therapeutic modalities.

Selenite-induced cataract has been proven to be a rapid and convenient animal model for cataract.[22,23] Several biochemical processes such as oxidative stress, altered epithelial metabolism, calcium accumulation, crystalline precipitation and cytoskeletal loss occur during the development of selenite-induced cataract.[22,23]

In the present study, all (100%) of the rats receiving a single subcutaneous injection of sodium selenite (20 μmol/kg) on postpartum day 10th developed cataracts, changes in protein profiles of the lens. In addition, SDS-PAGE results showed changes in soluble proteins profiles.

The well-established concept that intraocular generation of the oxygen-free radicals initiate oxidative stress, resulting in cataract formation, was initially proposed by many laboratories,[24-26] and the role of oxidative stress in cataract development and the importance of antioxidants in prevention of cataract has been a subject of research in ophthalmology but there have been conflicting results.[10-18,25-30]

In the present study, we have focused on L-Cysteine and vitamin C in preventing cataract and its associated biochemical and eletrophoretic changes. Based on the results, subcutaneous injections of .05 μmol and 0.1 μmol L-cysteine prevented selenite-induced cataract in 75% and 80% of rats, respectively. Both concentrations of L-cystein were more effective than vitamin C in prevention of cataract but there was no statistically significant difference between the two concentrations of L-cystein. L-cystein group also had significantly higher total lens protein and soluble protein concentration compared with the group of rats that only received sodium selenite. In addition, L-cystein groups showed near to normal appearance of the bands of soluble lens proteins in gel electrophoresis.

The normal crystalline-clear lens is characterized by a uniquely high content of glutathione. Low content of glutathione is the first abnormal biochemical

change known to precede every known form of irreversible cataract formation.[31] L-cysteine is the rate-limiting substrate in glutathione biosynthesis in rat and human lenses,[32,33] and its uptake decreases 4-fold with aging.[33] Hence, increasing the L-cysteine pool is likely to increase the rate of glutathione synthesis. Physiological concentrations of L-cysteine are normally very low because of its participation in both anabolic and catabolic reactions.[34] Decreased free L-cysteine in the diabetic rat lens precedes hydration changes and vacuole formation.[35] Exogenous L-cysteine may be a pharmacologic means for delivering L-cysteine to cells, potentially preventing cataract formation. L-cysteine prodrugs has been effective in yielding higher glutathione levels in the cultured rat lens.[36] In addition, L-cysteine has an antioxidant effect[37] and this effect can potentially contribute to the prevention of oxidative damage of the lens in the cataract process. CySSME, an L-cysteine prodrug, has been effective in preventing acetaminophen- and naphthalene-induced cataract in mice and has maintained near-normal glutathione levels in lenses and livers of such treated animals.[38]

Vitamin C is another agent whose role in prevention of cataract has been a subject of research.[35,39-45] In the present study, subcutaneous injections of vitamin C prevented selenite- induced cataract in 50% of rats. Vitamin C also increased total protein concentration of the lens and soluble protein concentration compared to the group of rats that only received sodium selenite. In addition, vitamin C groups showed near to normal pattern of the bands of lens proteins in gel electrophoresis.

Several studies have shown a lower incidence of cataracts in population consuming greater amounts of vitamin C.[39,40] In the diabetic rat lens, it has been shown that lens ascorbate pool diminishes prior to lens hydration[35]. Also, it has been demonstrated that vitamin C protects the rat lens against oxidative damage under photochemical as well as ambient conditions.[42,43] It has also been shown that vitamin C protects the lens against oxidative stress, leading to cataract formation 30. The concentration of ascorbate in the occular tissues is relatively high in comparison to most other body tissues and its concentration is higher in the aqueous humor and the lens of the diurnals as compared to the nocturnals. Because the incidence of cataract is higher in the population living in areas with greater sun exposure, it has been proposed that this may be related to photocatalyzed conversion of molecular oxygen to its excited states of very high reactivity such as singlet oxygen, hydroxyl radical, superoxide, hydrogen peroxide by.[43] In addition, high ascorbate in the aqueous could also act as a filter, preventing penetration of UV light into the lens and thereby protecting the tissue against its direct adverse effects on the protein and nucleic acid structures.[44,45] In the light of such evidence, the effect of vitamin C on prevention of cataract can be elucidated and the results of the present study add to the growing body of evidence that suggests a possible beneficial effect of vitamin C in the prevention of cataract formation. In conclusion, this study demonstrates morphological, biochemical and electrophoretic changes of the lens in an experimental model of cataract and shows preventive effects of L-cysteine and vitamin C.

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Conflict of interest: None declared.

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