

EFFECTS OF ERDOSTEINE ON OXIDATIVE-ANTIOXIDATIVE EQUILIBRIUM AND ON CATARACT FORMATION IN RAT PUPS WITH SELENITE-INDUCED CATARACT

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Aim: To investigate whether erdosteine supplementation following selenite exposure affects oxidant-antioxidant equilibrium and prevents cataract formation in rat pups.

Methods: Thirty-nine Wistar-albino rat pups were divided into 3 groups. In Group 1 (n=16) only s.c saline was injected. In Group 2 (n=10) subcutaneous (s.c.) sodium selenite (30 nmol / g body weight) was injected on postpartum day 10. In Group 3 (n=13) s.c. sodium selenite (30 nmol/g body weight) were injected on postpartum day 10 and oral erdosteine (10 mg/kg body weight, daily for one week) was administered by gavage thereafter. The development of cataract was assessed weekly. The density of cataract was graded by slit-lamp biomicroscopy. On day 21, the blood was collected and lenses were removed. Oxidative stress index (OSI) and total antioxidant capacity (TAC) were determined in the lenses of the rat pups. Paraoxonase-1 (PON 1) activity was determined in the sera.

Results: All of the lenses of rat pups in Group 1 remained clean. All rat pups developed dense nuclear opacity in Group 2. Eight out of 13 rat pups developed slight nuclear opacity in Group 3. Differences among the groups were statistically significant (p<0.05). Group 2 lenses had higher mean OSI level than that of Group 3 lenses (p=0.003). Group 2 lenses lower mean TAC levels than that of Group 3 (not significant). Mean PON 1 level of Group 2 was lower than that of Group 3 (p<0.05).

Conclusion: Erdosteine diminishes the incidence of cataract due to its protection of the antioxidant defense system.

Key words: Antioxidant, erdosteine, paraoxonase-1, selenite cataract

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INTRODUCTION

Ostadalova was the first to report selenite-induced cataract formation mimics senile cataract in 1978 (1,2). A single subcutaneous injection of 19-30 µmol/ kg body weight of sodium selenite into 10-14-day-old-rat pup is adequate to produce selenite-induced cataract in a period of six days (3). Oxidative stress induced by free radicals triggers senile cataractogenesis (4). The sequential mechanism that causes selenite-induced cataract through calpain over-activation involves nuclear calcium accumulation, proteolysis of crystallins and cytoskeletal proteins, phase transition, and precipitation of crystallins (3). Oxidation of lens proteins is probably important in senile cataractogenesis.

Oxidative damage is suggested to contribute to selenite cataract formation process (2). Antioxidant agents such as vitamin C, caffeic acid phenethyl ester, and α -ketoglutarate were reported to prevent the oxidative stress, thus selenite-induced cataract formation (5-7).

Paraoxonase-1 (PON 1) is an enzyme with paraoxonase, aryl esterase, and diazoxonase activities (8). PON-1 protects LDL and HDL from oxidation induced by copper ions and free radical generators (9). PON 1 hydrolyzing activity of some activated phospholipids (10) and lipid peroxide products is associated with the protection (9). Furthermore, antioxidants preserve serum PON 1 level (11).

As far as we are aware, the effect of erdosteine on selenite-induced cataract formation has not been previously reported.

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The aim of the present study was to investigate whether erdosteine prevents selenite-induced cataract formation in rat pups. Furthermore, we have assessed the effects of erdosteine on the oxidative-antioxidative equilibrium in the lens and sera.

MATERIAL AND METHODS

Thirty-nine Wistar-albino rat pups were divided into 3 groups. The study was conducted in accordance with the ARVO Statement for Use of Animals in Ophthalmic and Vision Research and Guiding Principles in the Care and Use of Animals. The rat pups were housed with the mothers. The rat pups in Group 1, sham, (n=16) received only subcutaneous (s.c.) saline. In Group 2 (n=10), s.c. sodium selenite (30 nmol/g body weight, Sigma Chemical Co., St. Louis, MO, USA) was injected on postpartum day 10. The rat pups in Group 3 (n=13) received s.c. sodium selenite (30 nmol/g body weight) on postpartum day 10, and oral erdosteine (10 mg/kg body weight, Erdostin, Sandoz, Turkey) daily for one week by gavage thereafter. The pups were observed each day starting from the day their eyes opened (14-16 days after birth). The development of cataract was assessed weekly by slit-lamp biomicroscopy. One person (A.K.), who was blinded to the group, performed biomicroscopic examinations of the lenses. On postpartum day 21, adequate pupillary dilation of the pupils were obtained with tropicamid 0.5% and phenylephrine hydrochloride 2.5% and the development of cataract was assessed; a) Mature dense opacity involving the entire lens; b) Slight nuclear opacity; and c) Normal clear lens.

Analysis in Lenses

At the end of the trial, the rat pups were sacrificed and their encapsulated lenses were removed by posterior approach without any delay. The lenses were rinsed with physiological saline, blotted on filter paper, homogenized in pairs in 10 volumes of phosphate buffer (20 mmol, pH 7.4). The supernatant was obtained by centrifugation in glass tubes at 6000xg for 30 minutes and was used for measurements.

Analysis in Blood

The blood samples obtained from the Inferior Vena Cava of the rat pups were placed in separate heparinized polypropylene tubes. The harvested plasma was discharged following centrifugation (2000xg, +4 °C,

15 minutes) and stored at -80 °C for further analysis of PON 1 activity. PON 1 activity was measured spectrophotometrically. All preparation procedures were performed at +4 °C.

Measurement of total oxidant status (TOS)

TOS values of the lenses were measured by a most recently developed automated method (12). In this method, hydrophilic and lipophilic oxidants oxidize ferrous ion to ferric ion. The oxidation reaction is enhanced using glycerol and the produced ferric ion makes a stable colored complex with xylenol orange, a ferric dye. Hydrogen peroxide solution is used as assay standards. The assay has got excellent CV% values, less than 3%.

Measurement of total antioxidant capacity (TAC)

TAC of lenses were determined using a novel automated measurement method, developed by Erel (13). In this method, hydroxyl radical, which is the most potent biological radical, is produced. In this assay, ferrous ion solution, which is present in the Reagent 1, is mixed by hydrogen peroxide, which is present in the Reagent 2. The sequential produced radicals such as brown-colored dianisidiny radical cation, produced by the hydroxyl radical, are also potent radicals. In this assay, antioxidative effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The assay has got excellent precision values, which are lower than 3%. The results are expressed as mmol Trolox equivalent / l.

Oxidative Stress Index (OSI)

The percent ratio of the TOS of lens to the TAC of lens gave the OSI of the lens, an indicator of the degree of oxidative stress [(TOS / TAC) x 100] (14-17).

Measurement of paraoxonase-1 (PON 1) activity

The rate of paraoxon hydrolysis (diethyl-p-nitrophenylphosphate) was measured by monitoring the increase of absorbency at 412 nm at 37 °C. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was 17000 M⁻¹ cm⁻¹ (18). PON 1 activity was expressed as U L⁻¹ serum.

Statistical Analysis

Statistical analyses were performed using

SPSS software (Version 13.0, SPSS, Inc, Chicago, Ill). One-way ANOVA and TUKEY tests were applied on the data shown normal distribution. Additionally, the Kruskal-Wallis and Mann-Whitney-U tests were used as indicated for the data showed abnormal distribution. Categorical variables were compared using the chi-squared test. The results were expressed as means \pm SD. *p* values less than 0.05 were considered to be significant.

RESULTS

All of the lenses of rat pups in Group 1 remained clean. All rat pups developed dense nuclear opacity in Group 2. Eight out of 13 rat pups developed slight nuclear opacity in Group 3. The other 5 rat pups in Group 3 had clear lenses. Differences among the groups were statistically significant ($p < 0.05$).

The mean OSI level in Group 2 (7.04 ± 1.19 Arbitrary Unit (AU)) was significantly higher than the mean OSI levels in Group 1 (5.08 ± 0.87 AU) and Group 3 (5.1 ± 0.84 AU) ($p = 0.003$, for both). The mean TAC level in Group 2 (4.17 ± 0.41 mmol Trolox Eqv / L) was lower than the mean TAC levels in Group 1 (4.66 ± 0.43 mmol Trolox Eqv / L) and Group 3 (4.46 ± 0.32 mmol Trolox Eqv / L), but the difference was not statistically significant. Because of the abnormal distribution in the data of PON 1, non-parametric Kruskal-Wallis and Mann-Whitney U tests were employed for the analysis. The mean PON 1 level in Group 2 (10.82 ± 29.29 U L⁻¹ serum) was significantly lower than the mean PON 1 levels in Group 1 (40.5 ± 92.86 U L⁻¹ serum) and Group 3 (32.31 ± 42.89 U L⁻¹ serum) ($p < 0.05$, for both).

DISCUSSION

Cataract is a major cause of severe visual impairment, even of blindness worldwide. Despite many experiments on the pharmacological agents to prevent cataractogenesis, surgical removal still remains the only effective treatment technique for senile cataract.

Selenite-induced cataract formation is a convenient, rapid, and reproducible experimental cataract model (1,3). The role of oxidative stress in selenite-induced cataract formation has been previously reported (5,19). Selenite causes oxidation of the components of the cell, including the proteins, and forms covalent linkage with protein sulfhydryl (3). Lipid peroxidation occurs in lenses, and hydrogen peroxide formation occurs in

aqueous humor (20). As the result of both the ion pump damage and the disturbance of the electrolytic balance, intracellular calcium increases. The calcium activates calcium dependent protease calpain, which hydrolyzes intracellular proteins. In the end, lens opacity occurs, and the activities of antioxidant enzymes decrease (3). The experimental model of selenite-induced cataract formation is similar to senile cataract formation in many ways: Vesicle formation, increased calcium, insoluble protein, proteolysis, and decreased water-soluble proteins. On the other hand, contrary to senile cataract, high-molecular-weight covalent aggregates or increase in disulfide formation does not occur in selenite cataract. Selenite cataract is dominated by rapid calpain-induced proteolytic precipitation, whereas human cataract occurs as a result of exposure to oxidative stress for a long period of time (3).

Numerous medical treatment methods were employed to prevent selenite-induced cataractogenesis, including Vitamin C, caffeic acid phenethyl ester, alpha-ketoglutarate, Ginkgo biloba, quercetin, propolis, and non-steroidal anti-inflammatory drugs (NSAIDs) (5-7,19,21,22). Among NSAIDs, the antioxidant effects of naproxen and diclofenac in selenite-induced cataractogenesis have been shown (19,21). Naproxen acts as an antioxidant in photocatalysed lens lipid peroxidation (23).

Erdosteine is a NSAID. It is a potent antioxidant and mucolytic pharmacological agent (24,25). Erdosteine inhibits lipid peroxidation (25). The pharmacological agent, therefore, decreases malondialdehyde (MDA), which is the end product of lipid peroxidation (24). Furthermore, erdosteine maintains free radical scavenging properties (25). Therefore, we hypothesized that erdosteine would prevent selenite-induced cataractogenesis in rat pups.

In the present study, the incidence of selenite-induced cataract decreased probably because of the diminished oxidative stress due to erdosteine. The lenses of 5 out of 13 rat pups remained clear at the end of the trial in Group 3.

In the current study, the mean PON 1 level in Group 2 was significantly lower than the mean PON 1 levels in the other groups ($p < 0.05$, for both). The lower mean PON 1 level in Group 2 was due to oxidative stress of selenite. We observed that erdosteine was effective in Group 3 because it caused an increase in mean PON 1 level in the group

when compared with the PON 1 level in Group 2. On the other hand, the mean OSI level in Group 2 was significantly higher than the mean OSI levels in Group 1 and Group 3 ($p=0.003$, for both). The higher mean OSI level in Group 2 was due to the oxidative status that selenite administration caused. Although the differences were not statistically significant, the mean TAC level in Group 2 was lower than the mean TAC levels in Group 1 and Group 3. Higher OSI and lower TAC levels in the lenses in Group 2 supported the antioxidant effect of erdosteine supplementation. It is plausible that erdosteine supplementation preserves oxidant-antioxidant equilibrium in both lenses and sera of rat.

To conclude, we investigated the effect of erdosteine on selenite-induced cataract formation in rat pups for the first time, and found out that erdosteine prevents the development of selenite induced cataract formation in rats probably due to its protection of the antioxidant defense system. Further studies should be encouraged to determine how to apply the pharmacological agent to humans.

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