Deferoxamine Effect on Selenite-Induced Cataract Formation in Rats
Zaiqi Wang, John L. Hess, and G. E. Bunce

A single subcutaneous dose of 30 nmol of sodium selenite per gram of body weight in 13-day-old rats resulted in posterior subcapsular cataract (PSC) after 24 hr and bilateral nuclear cataracts at 72-96 hr. Within 24 hr of treatment, a 60% decrease in lens glutathione was seen. A loss of calcium homeostasis observed by 48 hr resulted in increased lens calcium (4 μmol/g dry weight), which accompanied nuclear opacification. The iron chelator, deferoxamine (DF), was evaluated as a potential protective agent against these selenite-induced changes. Three doses each consisting of 1.1 μmol DF/g body weight were administered during the initial 24 hr of selenite exposure. Within 96 hr, all lenses from animals treated only with DF remained transparent, but 50% of these lenses showed cortical cataract at 3 wk postinjection. Concurrent administration of DF and selenite protected 80% of rats against PSC after 48 hr and 25% against nuclear cataract after 96 hr. No elevation in lens calcium occurred in the protected lenses. An additional 20% of animals were not protected fully but showed substantially less nuclear opacity than with selenite alone. They had a significant but moderate increase in lens calcium. After 3 wk (animal age, 35–40 d), cataract appeared in these "protected" lenses involving both the nucleus and cortex and loss of ion homeostasis. The glutathione content remained lower in lenses from animals treated with both selenite and DF compared with those from selenite-treated animals. In conclusion, DF may protect lenses from early oxidative damage from selenite exposure, and delay the response of the lens to subsequent metabolic injury caused by exposure to selenite. However, high doses of DF provoked cataract formation in a longer time frame. Invest Ophthalmol Vis Sci 33:2511-2519, 1992

The cause of selenium-induced cataract has been investigated by several laboratories during the past decade, but the precise pathway of selenium toxicity remains unresolved. A recent study suggests that active oxygen can be generated by the reaction of selenite with reduced glutathione. Lipid peroxidation and a 2.6-fold increase in aqueous humor hydrogen peroxide have been reported to accompany formation of selenite-induced nuclear cataract. Under normal circumstances, lens hydrogen peroxide is scavenged by glutathione peroxidase and catalase. However, total lens glutathione declines by approximately 70% in lenses from selenite-treated rats. As a consequence of the decreased glutathione, accumulated hydrogen peroxide would be available for interaction with free iron or other transition metals (Fenton reaction) to form cytotoxic hydroxyl radicals. Superoxide anion generated by the reaction of glutathione with selenite also might be a source of hydroxyl radical. Such radicals are thought to be involved in lipid peroxidation of the cell membrane, oxidation of proteins, and DNA damage. Recent studies in our laboratory show that increased DNA damage (strand breaks) is an early response to a single subcutaneous injection of selenite in rat lens.

If selenite toxicity were mediated by reactive oxygen species, then the chelation of iron might reduce formation of cytotoxic free radicals in the lens. Iron chelators have been shown to reduce iron overload-induced tissue damage in the cardiovascular system and to protect against ischemia-induced brain damage. Furthermore, iron was reported to be increased in lenses from selenite-treated rats. Therefore, the effect of the iron chelator, deferoxamine (DF), on selenite-induced cataract was quantified in this study.

Materials and Methods

Animal Treatments

All procedures involving animals conformed to the ARVO Resolution on the Use of Animals in Research.
and the “Guide for the Care and Use of Laboratory Animals” (DHEW, NIH 86-23). Stock solutions of 490 mmol/l DF and 20 mmol/l sodium selenite were prepared in 0.9% NaCl. The experiment was initiated by subcutaneous injection of the selenite (30 nmol per gram of body weight) into 13-day-old Sprague-Dawley rats. Within the first 24 hr, these animal received three equivalent intraperitoneal injections (750 μg/g body weight) of DF (total dose of DF, 3.35 μmol/g body weight). Body weight and lens growth were monitored. Control animals from the same litter were not injected. In all experiments, age-matched animals were the source of the control lenses.

Reagents

Sodium selenite was obtained from Aldrich (Milwaukee, WI); DF, as the methylsulfonate ester, and all other reagents were from Sigma (St. Louis, MO) unless otherwise noted.

Ion Analyses

At appropriate times, control and treated rats were decapitated. Their lenses were removed with the capsule intact, quickly washed with 50 μl of 0.5 mmol/l EGTA, and blotted on filter paper. The lenses were dried at 100°C for 48 hr, and two lenses/sample were digested alternately with concentrated nitric acid (Ultrapure grade; Baker, Phillipsburg, NJ) and H₂O₂ 30% (Fisher, Springfield, NJ) at 200°C to dryness. This digestion was repeated, and the residual salts were dissolved in HCl 10%.

Simultaneous measurement of calcium, potassium, and sodium was accomplished using inductively coupled plasma analysis on the Jarrell-Ash (Franklin, MA) ICAP 9000 instrument in the Soil Testing and Plant Analysis Laboratory (VPI & SU, Blacksburg, VA).

Glutathione Assay

The lenses were removed immediately after the animal was decapitated. They were weighed and extracted with a solution (0.5 ml/lens) containing 0.9 mol/l perchloric acid and 0.05 mol/l phosphoric acid. Glutathione was analyzed on a 1:50 dilution of the extract using the glutathione reductase dependent cycling assay.

Photography

The lenses were kept in prewarmed Hank’s TC 199 (30°–34°C) medium during microscopic evaluation and photography. Both light- and dark-field photographs were taken with an Olympus OM-2S camera attached to an Olympus dissecting microscope equipped with a below-stage illuminator (Model SZH-ILLD; Tokyo Japan).

Statistical Methods

All data are expressed as the means of values from the same litter. In reporting lens weight and reduced glutathione (GSH) content, the means of at least three separate experiments are reported. The standard error for these means was less than 10% of the mean. Analytic data for ion content were evaluated over time using standard linear least-squares or exponential regression analysis on the means of values from animals of the same litter. Although the regression lines revealed trends in the change of lens ion content, student t-tests were applied only to specific time comparisons. Effects of treatments on animal weights were compared using a student t-test comparison of slopes of the linear-regression analysis of weights of all animals aged 18–35 d.

Results

Lens Transparency and Size

In agreement with previous studies, treatment with selenite alone was followed by formation of posterior subcapsular cataract (PSC) within 12–48 hr and nuclear cataract (NC) within 72–96 hr. We observed that a dose of DF of 4.3 μmol/g body weight approached the lethal dose for 50% of the animals, but we saw no deaths at the 3.35 μmol/g body weight, the level used in these experiments. The relationship between the dose of DF and the rate of protection of PSC and NC (Fig. 1) indicated that, at a DF dose of 3.35 μmol/g body weight, there was 80% protection against the appearance of PSC after 48 hr and 25% protection against NC after 96 hr. Of the remaining 75% of NC lenses, 20% had less severe cataract than observed with selenite treatment alone. The other 55% had typical nuclear opacity. At the age of 35–40 d (3 wk postinjection), cataracts involving both the nucleus and cortex appeared in these “protected” lenses. We also observed a 50% occurrence of exclusively cortical cataract in animals 3 wk after treatment with only DF. Slit-lamp evaluation of rats 48 and 96 hr after treatment confirmed these observations in lenses removed from animals.

The lenses were photographed at these different stages of cataract development (Fig. 2). The number of rats observed are indicated by the values in parentheses. At least 28 lenses were examined in all groups, and 244 lenses were examined in the group treated with selenite and DF. Because treatments were based on comparisons within litters of 12 animals, usually 3–6 animals/litter were used for each treatment.
DEFEROXAMINE EFFECTS ON CATARACT / Wang er ol 2513

Fig. 1. Effect of DF dose on the occurrence of cataracts in sele-
nite-treated rats. Rats were injected with 30 nmol selenite/g body
weight with the first of three injections of DF at the designated dose.
Posterior subcapsular cataract was evaluated 48 hr after the treat-
ment. Nuclear cataract was evaluated after 96 hr. At least six ani-
mals from two litters were compared with age-matched controls
that had clear lenses.

Significant inhibition of lens growth (estimated from lens dry weight) was observed in selenite- and selenite-and-DF-treated rats (Fig. 3). After 3 wk, massive swelling and increased lens weight occurred only in lenses from animals treated with both selenite and DF (data not shown). This swelling correlated with the continued loss of ion homeostasis in these lenses (Figs. 4-6).

Effects on Lens Ion Homeostasis

In lenses from selenite-treated rats, calcium significantly increased during 48–96 hr after injection of selenite (Fig. 4A). Considered as one group, the lenses from the animals treated simultaneously with selenite and DF tended to have more Ca^{2+} than did those from control animals, but this difference was not significant (Fig. 4A). However, this group (selenite and DF treatment) consisted of three classes of lenses with the following different calcium concentrations (mean ± standard error): clear lens, 0.96 ± 0.01 μmol/g dry weight; partial protection, 1.49 ± 0.2 μmol/g dry weight; and nuclear cataract, 4.3 ± 0.6 μmol/g dry weight.

When the lenses in the selenite-treated animals began to grow again, the Ca^{2+} concentration returned to normal values (Fig. 4B). In the animals treated with both selenite and DF, however, Ca^{2+} accumulation continued; its content became 10–20-fold that of control concentrations (Fig. 4B).

Rats treated with both selenite and DF 18 d posttreatment also had a loss of Na^{+} homeostasis (Fig. 5) and accompanying loss of K^{+} homeostasis (Fig. 6). Significant differences compared with control animals were not observed in concentrations of either Na^{+} or K^{+} in lenses from animals treated with selenite alone or DF alone (Figs. 5, 6).

Effect on Lens Glutathione

The expected significant loss of total glutathione in these lenses occurred after injection of selenite. Concurrent treatment with DF did not affect the total glutathione concentration up to 96 hr after administration of selenite, but it prevented the expected recovery of glutathione that occurs in lenses from selenite-treated rats (Fig. 7). The DF treatment alone caused an initial 30% loss of lens glutathione concentration that returned to control values by 29 d after treatment.

Other Effects of DF Treatment

All rats that received DF injections with or without selenite had hair loss over their entire bodies except the crowns of their heads. The loss occurred over a 48-hr period, and the skin condition was normal without any lesions. Animal behavior was similar between the control and treated animals. Regrowth of hair began by day 7 after DF treatment and continued until a normal coat was restored.

Body weight gain was diminished significantly in selenite-treated animals. By 23 d after treatment, control animals weighed 160 ± 14 g compared with 110 ± 8 g for the treated animals. These values were determined from the regression analysis of at least 50 animals for each treatment over the 39 d period of the experiment (Fig. 8). For comparison, the mean ± the standard deviation for five control animals at day 39 was 157 ± 6 g. As indicated in the legend for this figure, these weight gains were significantly different (by student t-test comparison of the slopes of these lines). By similar evaluation, rats treated with both DF and selenite weighed (105 ± 7 g) significantly less than control animals. Those treated with only DF weighed 145 ± 12 g, and the weight gain was not different from the untreated control animals.

Discussion

The initial report that cataract appeared in virtually all suckling rats within 3–4 d after subcutaneous sodium selenite injection of 20–30 nmol/g body weight has been verified by many investigators. The phenomenon described by these workers was a dense nuclear opacity. Subsequent biochemical, microscopic, and histologic examinations showed a com-
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* values in parentheses indicate number of rats.

Fig. 2. Appearance of typical lenses from control animals and rats treated with selenite and DF (3.35 μmol DF/g body weight). Appearances at four key times are shown. Bright field (left) and dark field (right) exposures are provided. The length of the grid line is 3.3 mm. For those lenses treated with DF and (+)Se, (+)DF, we present typical views of the cataractous ("unprotected") lenses. The numbers in parentheses represents the total number of rats evaluated for each treatment.
plex series of events that yield multiple forms of opacities. The earliest histologic changes were seen 5 hr postinjection and consisted of nuclear fragmentation and suppression of mitosis. Appearance of PSC 12–30 hr postinjection correlated with the appearance of vacuoles. The dense central NC that appeared at 3–5 d postinjection was preceded by a change in refraction in the perinuclear region. Vacuolization of the cortical area was observed 6–15 d postinjection. At 15–30 d postinjection, cortical opacities were detected, which, at the histologic level, showed wrinkling of the capsule and multilayered abnormal epithelium. The lens bow appeared abnormal, and there was total loss of meridional row structure. Between 1–2 months postinjection, the cortical opacity cleared, and the normal meridional row structure was recovered. However, the nuclear opacity was permanent. Others concluded that the cortical and nuclear opacities arose by different mechanisms. The nuclear opacity was thought to be a direct consequence of calcium-stimulated proteolytic attack of calpain II on the lens crystallins and membrane proteins; the cortical opacity appeared to be a result of an episode of abnormal fibrogenesis. Both, however, along with PSC, may have a common origin in the selenite-stimulated generation of oxidant stress. This study was undertaken...
to determine the benefit of administering the iron chelator, deferoxamine, as an agent to diminish the level of oxidant production.

The administration of DF was highly effective (80%) in preventing PSC in selenite-treated rats. This response suggests that production of oxidant species with resultant localized osmotic swelling is an important factor during the earliest stages of selenite toxicity. The drug less effectively prevented NC, which appeared 3–5 d postinjection. Only 25% of the selenite and DF-treated rats had clear lenses, although another 20% had cataracts that were much reduced in size compared with the usual response. The benefits associated with concurrent DF administration were transient; both nuclear and cortical regions became opaque in the selenite and DF-treated rats 3 wk postinjection. Moreover, cortical cataracts were apparent at the same time in 50% of rats injected with DF alone.

Fig. 5. Effect of selenite and DF on lens sodium content. Data points represent the mean of at least three samples, two lenses per sample. Each value represents a different litter. After 96 hr, the sodium content only in lenses from (+)Se, (+)DF-treated rats (•••••) was significantly different from controls (□—□). After 18 d, this difference had increased to about 10 times the control level.

Fig. 6. Effect of selenite and DF on lens potassium. Data points represent the mean of at least three samples, two lenses per sample. Each data point represents a different litter. Only after 18 d was the potassium content in lenses from (+)Se, (+)DF-treated animals (•••••) significantly less than those from controls (□—□).
Cataracts have been reported in patients undergoing DF treatment for thalassemia.\textsuperscript{18} The major route of selenite metabolism is reduction by glutathione to H\textsubscript{2}Se, which creates a demand for nicotinamide adenine dinucleotide phosphate to renew GSH from oxidized glutathione (GSSG). We found that lenses from selenite-injected animals show a marked stimulation in hexose monophosphate shunt activity and that this pathway is fully operational and capable of a further increase in rate.\textsuperscript{19} The concentration of total lens glutathione drops to approximately 25–30\% of normal within 12–24 hr post-injection and requires 20–25 d to recover completely despite the observation that a single cataractogenic dose of selenite appears to be cleared from the lens within 1 wk. Presumably, glutathione is lost by passive diffusion of GSSG. Administration of DF did not prevent the usual selenite-induced decline in total glutathione. Moreover, DF appeared to inhibit the recovery in lens glutathione that began 15 d postinjection. This drug alone caused a 30\% decline in glutathione that persisted for approximately 20 d. These results suggest that DF itself may have stimulated the production of oxidant species capable of reacting with glutathione. Under certain circumstances, DF also may act as a prooxidant, especially in the presence of an abundance of ascorbic acid.\textsuperscript{20} Recent studies also found that DF may increase the Fe\textsuperscript{2+}/Fe\textsuperscript{3+} ratio in a cell and potentially favor the Fenton reaction-dependent production of hydroxyl radicals.\textsuperscript{21} Others\textsuperscript{22} reported protection of lenses from diquat-induced cataract with a DF–manganese (III) complex. They noticed, however, that treatments with DF alone did not effectively prevent but rather enhanced bleomycin- or doxorubicin-induced cataracts.

The lack of protection by DF also may be related to the toxic effect of DF itself toward lenses. Alone DF caused cortical opacities in 50\% of the lenses 3 wk after treatment (Fig. 2). In lenses from DF-treated rats, there was approximately a 30\% loss of lens total GSH; this concentration recovered only slowly after the initial injection (Fig. 7). There was, however, no significant loss of ion homeostasis in these lenses (Figs. 4–6). Because iron is a requirement for GSH synthesis, the loss of lens GSH may be caused by chelation of iron by the drug. This requirement is consistent with the high \(\gamma\)-glutamylcysteine synthetase activity reported to occur in iron-loaded mice.\textsuperscript{23} There was no recovery of glutathione in the lenses from animals treated with both selenite and DF even after 4 wk. Ocular toxicity of high doses of DF occurred in patients receiving intravenous DF therapy.\textsuperscript{24} The authors suggest this response may be related to the chelation effects of DF on trace mineral ions particularly copper, zinc, cobalt, and nickel. Another study\textsuperscript{25} suggested that the neuroophthalmic toxicity of high-dose DF therapy in humans correlated with a DF-dependent increase in "loosely bound" copper in cerebrospinal fluid and substantiated that ocular toxicity may not necessarily be related to chelation effects of DF with iron. Furthermore, DF at the concentrations of 20–100 \(\mu\)M can inhibit cell proliferation in vitro and in vivo; it may do so by depriving the cells of iron and
inhibiting the enzyme ribonucleoside diphosphate reductase. This effect may have caused the inhibition of lens growth in DF and selenite-treated rats we reported.

An increase in lens calcium begins by 48 hr after selenite injection and reaches its peak of three- to fivefold 24-48 hr later. It is well established that this increase provides sufficient free calcium ion to activate the proteolytic enzyme calpain II. Simultaneous administration of DF and selenite diminished the amount of excess calcium accumulation by approximately 40% 4 d postinjection. By 3 wk postinjection, however, lens calcium in the DF and selenite-treated group was elevated to approximately tenfold above normal. This elevation coincided with the emergence of extensive regions of both cortical and nuclear cataract in these animals. Alone, DF had no effect on lens calcium, although cortical cataracts were noticed in about 50% of the animals. These results are consistent with the ability of DF to diminish production of oxidants during the earliest period of selenite challenge but provide additional evidence that the long-term effects of coincident exposure are severely debilitating to the lens. Individual treatment with selenite or DF did not significantly affect sodium and potassium homeostasis in lenses. Although simultaneous DF administration with selenite did not alter the lens concentrations of these ions during the first 48 hr, there was a trend toward increased Na+. By 96 hr postinjection, however, monovalent cation imbalance was apparent, and by 21 d, it was significantly different from lenses of control or selenite- or DF-treated animals (Figs. 4-6).

Although, in this study, we observed restricted weight gain in selenite-treated preweaning rats, this effect was not documented previously over the 3 wk after a single injection (Fig. 8). Furthermore, we did not find any reports in the literature of the massive hair loss from the preweaning rat that we observed in rats treated with DF. The mechanisms by which selenite affected limited growth in young animals or DF caused extensive hair loss remain unknown. The DF dose in these experiments was approximately threefold greater than that normally used in human chelation therapy.

In conclusion, our studies show that DF protects the rat lens against certain responses occurring during the initial stages after selenite exposure (PSC and an increase in lens Ca2+) and only partially diminishes the severity of NC formation at 72-96 hr postinjection. This protection suggests that initial oxidative events may give rise to this lens pathology because DF-induced iron chelation has potential benefit in protecting against oxidative stress. However, the high doses of DF required to achieve protection from the selenite stress caused cortical cataracts in 50% of treated animals 18 d after treatment. Furthermore, these doses of DF in the presence of selenite resulted in massive lens degeneration after 3 wk. The potential for long-term toxic effects from DF treatment requires additional study.

Key words: lens, cataract, selenite, deferoxamine, ion homeostasis

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References