Cataractous Changes in Rat Lens Following Cigarette Smoke Exposure Is Prevented by Parenteral Deferoxamine Therapy

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Objectives: To test whether iron accumulation in the lens following cigarette smoke exposure is the principal mechanism in smoke-related cataractogenesis and to assess the possible protective effect of deferoxamine mesylate treatment against lenticular degeneration with in vivo exposure to cigarette smoke.

Methods: Thirty-two male Wistar rats were randomly divided into 4 equal groups. Groups 3 and 4 rats were exposed to cigarette smoke for 1 hour each day for 90 consecutive days, and groups 1 and 2 rats were treated in a similar manner but exposed only to room air. In addition, deferoxamine was given subcutaneously to groups 2 and 4 rats. Both eyes of all the animals were then enucleated and 1 eye prepared for histopathological examination. The fellow eye was used to measure iron, calcium, zinc, and copper levels.

Results: Significantly higher iron and calcium and lower zinc levels were observed in the lenses of group 3 rats compared with those in the other groups. Similar comparisons performed between groups 1 and 2, 1 and 4, and 2 and 4 did not show any significant difference. Copper concentrations did not differ between groups. Distinct histopathological changes in the anterior lens epithelium, such as hyperplasia, hypertrophy, and epithelial multilayering, and the presence of swollen epithelial cells overlying the posterior lens capsule, observed in group 3 rats, were not present in the other groups.

Conclusions: Cataractogenesis following cigarette smoke exposure in rats was associated with the accumulation of iron, and concurrent deferoxamine therapy prevented such cataract formation.

Clinical Relevance: Our results may apply to human cataract formation associated with cigarette smoking, so such pathogenesis may be prevented by concurrent parenteral deferoxamine treatment. Clinical studies are needed, however, to determine the value of this suggestion.


ANY epidemiological studies1,2 have revealed that cigarette smoking is a strong risk factor for cataract development in both men and women. The most probable mechanism by which cigarette smoke contributes to cataractogenesis is oxidative damage3,4 because cigarette smoke–exposed tissues contain large amounts of reactive oxygen species (ROS) and metals. Indeed, the accumulation of ROS in the eye lens may contribute to cataractogenesis.5,6 Reactive oxygen species in living tissues can be generated by 2 routes. They can be produced photodynamically through the mediation of sensitizer molecules that have been excited to higher electronic states by light absorption and through the Fenton reaction.7,8 Substantial lenticular damage and the generation of ROS have been shown3 in smoke-exposed rat lenses, even in the absence of light. Various metals that undergo univalent redox reactions can participate in the enzymatic and nonenzymatic oxidation and peroxidation of biological molecules and, through the Fenton reaction,10 can reduce oxygen to more toxic oxygen free radicals—hydrogen peroxide and hydroxyl radical. Of these metals, manganese, iron, cobalt, and copper are of biological importance, but iron is the most effective catalyst in these oxidative processes.11 In vivo exposure to ambient cigarette smoke has been reported12 to increase the iron concentration and to decrease the zinc concentration in rat lenses. The decrease in zinc concentration after smoke exposure is indirect evidence of oxidative damage because zinc is a cofactor of several antioxidant enzymes and also has antioxidant properties.13 The present study was planned to determine the possible protective effect of the iron-chelating agent, deferoxamine mesylate, against cataract formation fol-
MATERIALS AND METHODS

Thirty-two male Wistar rats (body weight, 200-250 g; age, 10-12 weeks) were randomly divided equally into 4 groups. The rats were housed in stainless-steel wire cages except during smoke exposure and fed with standard rat chow and tap water ad libitum. The study was carried out according to the Association for Research in Vision and Ophthalmology Resolution for the Use of Animals in Research. Group 1 was sham-exposed, group 2 was sham-exposed plus given deferoxamine subcutaneously, group 3 was exposed to cigarette smoke, and group 4 was exposed to cigarette smoke plus given deferoxamine subcutaneously. Cigarettes that are manufactured from a blend of Turkish tobacco (Maltepe; Tekel Cigarette Factory, Istanbul, Turkey) were used in the study. Groups 3 and 4 rats were exposed to cigarette smoke for 1 hour each day for 90 consecutive days using a smoking machine. Groups 1 and 2 rats were restrained in identical chambers but exposed only to room air. Immediately before either smoke or sham exposure, deferoxamine mesylate (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) was given to groups 2 and 4 rats subcutaneously in a dose of 10 mg/kg of body weight for 90 consecutive days. The system has been described in detail in a previous study. One volume of cigarette smoke was mixed with 9 volumes of room air in the dilution chamber using rotating fans. Duration of each exposure was set at 1 hour, and rats were placed in the exposure chambers (20 L) and inhaled 10% cigarette smoke. Each filter cigarette was burned for 8 to 10 minutes, and 18 to 21 cigarettes were used each day. Thus, a mean of 2½ cigarettes was used per rat each day.

Both eyes of all rats were enucleated following dilation of the pupils by administering topical tropicamide drops (4%) under high ether anesthesia after the last exposure and kept, without cooling, in an insulated container until the time of preparation. The anterior lens epithelium was rolled (without disturbing its capsular integrity) after cutting the suspensory ligaments, taking care to avoid contamination from neighboring tissues and environmental sources. One of the freshly dissected lenses was rolled in filter paper to remove all adherent vitreous and iris and then dried at 80°C for 24 hours. The other lens of each animal was prepared for histopathological examination. The dried lens was weighed to the nearest 0.1 mg using an electronic balance (Sartorius Basic Electrobalance, model BA1105; Sartorius Corporation, Gottingen, Germany) and transferred to a glass vial prewashed with a trace element of free nitric acid. After the lens was cleaned with deionized water to remove residual nitric acid, it was digested by heating to more than 140°C in a mixture of concentrated nitric and perchloric acids (in a volume ratio of 5:1) until the organic matrix was completely dissolved. The mouth of the tube was covered with paraffin and stored at more than 4°C until analysis could be performed. The flame atomic absorption technique (Spectra AA, model 400; Varian Australia Pty Ltd, Mulgrave, Australia) was used to measure tissue metal levels. The element content was expressed as micrograms per gram of dry tissue weight. Before tissue measurements, standard graphics that fit those provided by the manufacturer were prepared for iron, zinc, copper, and calcium. The values obtained from these standard works were fitted to the graphics provided by the manufacturer so that blank tissue measurements gave 0 results for all of the metals. The lenses were observed under an operating microscope before enucleation.

The fellow lens of each rat was fixed in 10% buffered paraformaldehyde. Tissue specimens were prepared with an autotechnicon (tissue processor) (model HMP 300; Carl Zeiss Corporation, Oberkochen, Germany) and then embedded in paraffin, with special care taken to vertically orient the lens in the paraffin block. The anterior capsule surface was then identified, and the section lines were oriented perpendicular to the anterior capsule. Five slices of 5-µm thickness were prepared for each lens using a microtome and stained with hematoxylin-eosin. The specimens were observed and photographed using a photomicroscope (Axiolab model; Carl Zeiss Corporation). The specimens were masked to prevent bias during their examination.

The results were expressed as means ± SDs. A Kruskal-Wallis 1-way analysis of variance test was used for statistical comparisons. Statistical significance was $P<.05$.

RESULTS

Significantly higher lenticular iron and calcium and lower zinc levels were observed in group 3 rats than in other groups. Comparisons performed between groups 1 and 2, 1 and 4, and 2 and 4 did not reveal any significant difference in lenticular iron, calcium, and zinc concentrations. Copper concentrations of the lenses did not show any significant difference among groups (Table). Anterior lens epithelial cells were unilayered and composed of low cuboidal and cylindrical type of epithelium in group 1 and 2 rats, and no epithelial cell was observed overlying the lens capsule behind the equator of the lens (Figure 1 and Figure 2). Epithelial cells had a fusiform and scanty cytoplasm with an oval nucleus in all the analyzed specimens from groups 1 and 2 (Figure 1). The arrangement of lens epithelial cells of group 3 animals, however, showed substantially different features compared with controls. Multilayering of anterior epithelial cells was present in all sections (2.4 ± 0.6 cell layers), and in 5 (12%) of the 40 slides, some areas of ulceration were present on the anterior lenticular epithelium. The cytoplasm had lost its fusiform appearance and become more abundant, and the nuclei were larger and more spherical compared with groups 1 and 2 rat lens cells (Figure 3). Furthermore, we observed edematous epithelial cells over the posterior lens capsule in all slices belonging to group 3 animals (Figure 4). On the other hand, group 4 animals had a different type of epithelium. Although the lenticular epithelium was unilayered in all slices, about 40% of the nuclei of the cells were more spherical compared with those of groups 1 and 2 (Figure 5).
In an experimental study, isolated capsulated rat lenses were incubated with cigarette smoke, and the cigarette smoke was observed to permeate the lens capsule and to opacify the lens in a dose-dependent manner. Antioxidants offered partial protection against this damage. This observation indicates that cigarette smoke could cause cataract formation through oxidative damage to the eye lens. Indeed, a large amount of ROS can be found in the smoke-exposed tissues. The possible sources of ROS in living tissues are photodynamics and a metal-induced Fenton reaction. The Fenton reaction is of interest because Shalini et al demonstrated that ROS production in smoke-exposed rat lenses was only slightly enhanced in the presence of light, and even in the absence of light, a large amount of ROS could be produced in such lenses. Thus, it may be assumed that a metal-catalyzed Fenton reaction stimulates the production of ROS in rat lenses following smoke exposure.

### Metal Concentrations in Rat Lenses

<table>
<thead>
<tr>
<th>Group</th>
<th>Iron</th>
<th>Calcium</th>
<th>Zinc</th>
<th>Copper</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (sham exposed; n = 8)</td>
<td>4.2 ± 1.1</td>
<td>2.6 ± 0.6</td>
<td>34.6 ± 8.8</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>2 (sham exposed plus given deferoxamine mesylate; n = 8)</td>
<td>3.7 ± 0.9</td>
<td>2.3 ± 0.6</td>
<td>34.8 ± 7.4</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>3 (smoke exposed; n = 8)</td>
<td>10.1 ± 2.6</td>
<td>4.2 ± 0.8</td>
<td>21.5 ± 3.5</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>4 (smoke exposed plus given deferoxamine; n = 8)</td>
<td>5.3 ± 1.2</td>
<td>2.8 ± 0.7</td>
<td>33.1 ± 7.0</td>
<td>0.8 ± 0.1</td>
</tr>
</tbody>
</table>

*Values are given as mean ± SD micrograms per gram of dry tissue weight. Statistical comparisons between groups 1 and 2, 1 and 4, and 2 and 4 did not reveal any significant differences in metal levels (P > .50 for all). Similar comparisons performed between groups 1 and 3, 2 and 3, and 3 and 4 showed significant differences (P < .05 for all).
Various metals can participate in the Fenton reaction, but iron and copper are of biological importance, and iron is the most effective in producing ROS.\textsuperscript{10,12} Cigarette smoke has been shown\textsuperscript{3,4} to contain large amounts of iron and copper. In addition, the gas phase of cigarette smoke was shown\textsuperscript{14,15} to induce iron release from ferritin molecules both in vivo and in vitro. In view of this evidence, Qian and Eaton\textsuperscript{16} suggest that tobacco smoke might contain substances capable of delocalizing iron. Ferritin-bound iron does not catalyze oxidative reactions, but once iron is released from the ferritin molecule, it can catalyze oxidative injury.\textsuperscript{1,7} Thus, 2 possible sources of iron accumulation in rat lenses following smoke exposure are the metal content of cigarette smoke and a cigarette smoke–induced release of iron from ferritin molecules. Which mechanism predominates, however, is not revealed by our current data. In a previous experiment,\textsuperscript{12} iron was shown to accumulate in rat lenses following smoke exposure, and this was associated with histopathological evidence of lens damage and a concurrent decrease in lenticular zinc levels. Evidence of iron-associated cataract formation was also found\textsuperscript{19} in the hereditary hyperferritinemia–cataract syndrome.

In the current experiment, we were able to show that concurrent deferoxamine treatment prevented iron accumulation and cataractous changes in the rat lenses following cigarette smoke exposure. Cataract formation following smoke exposure in our experiment most probably was mediated by oxidative injury because it was associated with a concurrent decrease in lenticular zinc levels. Concurrent deferoxamine supplementation prevented such a decrease in the zinc level in rat lenses, which is indirect evidence that the concurrent use of deferoxamine can prevent oxidative damage. Deferoxamine is a specific, high-affinity chelator of iron, and deferoxamine-chelated iron does not catalyze hydroxyl radical formation. Furthermore, the use of deferoxamine prevents iron-catalyzed lipid peroxidation.\textsuperscript{17} Although deferoxamine has relatively poor intracellular penetration, it reduces tissue iron content in part by chelating extracellular iron.\textsuperscript{17} The present study provides some evidence for the role of an iron-mediated Fenton reaction in smoke-related cataractogenesis in rat lenses. Indeed, some researchers\textsuperscript{16} think that elements in tobacco smoke damage tissues through reactions mediated by the (probably iron-dependent) formation of the hydroxyl radical.

Histopathological changes observed in the present study after cigarette smoke exposure (group 3 rats) may indicate cataractous changes because the proliferation of the anterior lenticular epithelium and multilayering is the characteristic histopathological finding\textsuperscript{10} in anterior subcapsular cataracts (ie, following inflammation, trauma, or atopic dermatitis). This type of cataract is a common response to many types of irritation.\textsuperscript{19} Furthermore, epithelial cells were noted on the posterior lens capsule in all slices from group 3 rats. The presence of epithelial cells on the lens, either normal in appearance or edematous, reflects a posterior migration of lenticular epithelium from the equator and is diagnostic of cataract.\textsuperscript{19} In addition to this migration, the epithelial cells on the posterior lens surface were swollen. This is another typical histopathological finding\textsuperscript{19} in cataractous lenses. We did not observe these cells in the lenses of smoke-exposed animals treated with deferoxamine. Thus, cataractous changes in rats exposed to cigarette smoke can be prevented by deferoxamine treatment.

Deferoxamine therapy also averts the elevation of calcium concentrations in rat lenses after exposure to cigarette smoke. Increased calcium concentrations observed in group 3 rats provide an additional clue of lens injury from in vivo exposure to cigarette smoke because elevated cytosolic calcium concentrations are recognized\textsuperscript{20} as a critical event in the initiation of cell injury. Thus, the prevention of calcium accumulation provides additional evidence that concurrent deferoxamine treatment prevented lens damage associated with cigarette smoke exposure.

We did not find any significant difference in the lens copper concentration among the groups. This finding is not surprising because copper has a dual function. It causes a Fenton reaction and is present in large concentrations in tobacco smoke, so it was expected to be present in a higher concentration in smoke-exposed animals than in other groups; on the other hand, copper is also essential for the activity of several antioxidant enzymes.\textsuperscript{21}

In this study, we provide some evidence supporting the role of iron-mediated oxidative injury in the pathogenesis of cigarette smoke–related cataract formation in rat lens. The treatment of smoke-exposed rats with an iron-chelating agent (deferoxamine) prevented cataractous changes and lens damage.

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CONCLUSIONS
REFERENCES


