Ultrastructural Study of Selenite-Induced Nuclear Cataracts

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The formation of the selenite-induced cataract was investigated by examining the ultrastructure of the cataract with transmission electron microscopy (TEM). A lacy, or honeycomb, appearance of the nuclear cataract seen by light microscopy was resolved by TEM to be due to the aggregation or precipitation of cytoplasmic material. Despite severe intracellular changes the fibers retained their close apposition to one another. These results are consistent with the hypothesis that a major lesion in selenite-induced nuclear cataracts is the formation of insoluble cytoplasmic aggregates. Invest Ophthalmol Vis Sci 25:751-757, 1984

Elevated doses of the essential trace mineral selenium cause nuclear cataracts in suckling rats.1-4 These cataracts develop within 4 days after oral or a single subcutaneous injection of sodium selenite, Na2SeO3.2-4 Selenium is a strong sulfhydryl oxidant in vitro,5 and, thus, the selenite-induced cataract may serve as a useful model for studies on the role of oxidant stressors in cataractogenesis.

Selenium-induced cataracts are predominantly nuclear, but slit-lamp studies have shown that swollen fibers and refractive changes appear in the lens cortex prior to the formation of the nuclear cataract.3 Very little is known about microscopic changes that occur during the formation of these lesions, although a previous light microscopic study6 showed the width of the nuclear lens fibers to be increased, whereas the width of the cortical fibers was decreased. Thus, the purpose of the present study was to investigate the mechanism of selenite-induced cataracts by examining the subcellular features of the cataract with transmission electron microscopy.

Materials and Methods

Pregnant Sprague-Dawley rats (DOBS, Charles River; Wilmington, MA) were housed individually and provided distilled water and Wayne F-6 Lab Blox ad libitum. After birth, litters were culled to 8 pups per litter. Pups in the experimental group received daily injections of sodium selenite (0.5 mg Se/kg) in saline from days 2–10 postpartum and control pups received daily injections of saline alone.

The pups were killed on day 11 postpartum by ether and the eyes immediately removed and placed in 2.5% glutaraldehyde buffered with sodium cacodylate (pH 7.2). Surgical and fixation procedures were carried out at room temperature. Microscopic changes due to the development of cold cataracts were distinguished easily from those produced by selenite. After a few minutes of submersion in the fixative, two incisions were made in the eye to facilitate fixation of the lens. The lenses were fixed intact for 3 days, and one of each pair of lenses was dissected into 10 specific pieces, which were washed in buffer and postfixed in 2% osmic acid. The second lens of each pair was left intact but otherwise processed in the same manner as the dissected lens. After postfixation, the tissues were washed in maleate buffer (pH 5.2) and stained en bloc with uranyl acetate. Subsequently, the tissues were dehydrated in ethanol and embedded in a low viscosity resin.7 One-micron-thick sections were cut from the resin blocks, placed on glass slides, and stained with toluidine blue for light microscopy observation.8 Thin sections were prepared with a diamond knife, picked up on formvar coated grids, and stained with uranyl acetate and lead citrate.10 Sections were viewed with a Philips 300 and Siemens 101 electron microscope.

Results

Light Microscopy

Light microscopic observations of lenses from pups in the control group showed that the tissue was composed of elongated cells (fibers) that were hexagonal in cross section. The lens fibers in the outer third of the lens stained a pale blue with toluidine blue. Progressively darker staining was seen in the more centrally
located fibers of the deep cortical region. Many darkly stained round spots were observed in each fiber. Similar spots are known to appear in the lenses of young rats and mice when the eye is cooled below body temperature, such as after enucleation or submersion of the eye tissues in cool solutions. The fibers of the lenticular nucleus were featureless and stained a homogenous dark purple or maroon (Fig. 1).

In the selenite-treated animals, the lens fibers of the cortical and deep cortical regions were similar to those seen in the control lenses. In contrast, the center of the lens showed pronounced changes after selenite. The whole nuclear region had a lacy or honeycomb-like pattern (Fig. 2) consisting of a network of darkly stained material surrounded by thick, darkly stained “membranes” separated by clear areas.

Transmission Electron Microscopy

The ultrastructural appearance of cells of the cortical regions of the control and cataractous lenses of the 11-day-old rat pups were similar. The fibers contained a granular cytoplasm of medium electron opacity and accumulations of more electron opaque material (Fig. 3). The accumulations consisted of a few round masses scattered throughout the cytoplasm with larger masses present in the peripheral cytoplasm. The larger masses were found along the bends in the cell membrane which give the lens fibers their characteristic hexagonal cross-section. These opacities were identical in size, shape, and position to the darkly staining cytoplasmic inclusions seen by light microscopy, and they are the cytoplasmic “cold spots” associated with the cooling of the lens.

In contrast to that seen in the cortical regions, the ultrastructural appearance of the nuclear region of the
control and cataractous lenses were strikingly different from each other. In the control lenses the nuclear region contained cells that were apposed closely and were filled with an electron opaque, featureless cytoplasm (Fig. 4), whereas the nuclear region of the cataractous lenses (Fig. 5) contained enlarged, irregularly shaped fibers, each of which had a lacy appearance. The honeycomb pattern formed by "darkly stained membranes," as seen by light microscopy, was resolved by electron microscopy into a layer of very electron opaque cytoplasm lying along the cell membrane (Fig. 6). Although the swollen lens fibers in most areas of the cataractous region appeared to have remained intact, the boundaries of some could not be seen clearly (Fig. 5). Low magnification electron micrographs taken of these regions suggested that some cell rupture and/or cell fusions had occurred. However, at higher magnification it was found that some of the observed "breaks" were actually the sites of gap junctions. These penta-laminate junctions had little electron opaque material on either side (Fig. 7).

The electron opaque cytoplasmic material also extended in an irregular network across the nuclear lens fibers. Generally this material was surrounded by a lighter, flocculent material (Fig. 8). However, in the central regions of the cataract (Fig. 6), and occasionally in more peripheral areas, lens fibers lacked the light flocculent material.

In the transition zone between the cataract and the noncataractous areas, three types of lens fibers were found: deep cortical fibers; transitional, possibly precataractous fibers; and cataractous lens fibers (Fig. 8). The first of these, the normal deep cortical fibers, contained a large number of electron opaque spots. These spots had a dark central core, and they were surrounded by only faint cytoplasmic material. Abutting these cells was the second type of fiber, the transitional lens fiber, which contained irregularly shaped aggregates of material of medium electron opacity. These aggregates were surrounded by a light flocculent material. The transitional fibers, in turn, abut the third fiber type, the cataractous lens fiber. The cataractous fibers contained large amounts of very electron-opaque material interspersed with small irregular patches of a much lighter material. Those cataractous fibers, located peripherally, contained only small areas of the pale material against a dark background, whereas those more centrally located contained a fine flocculent cytoplasm in which fine networks of very opaque cytoplasm appeared against a light background. In the most central regions, only opaque material remained in each lens fiber.

Although the cataracts in these 11-day-old rat pups generally were limited to the nuclear region, spurs of cataractous fibers 2–3-cells thick extended into the cortical regions (Fig. 9). These radiated from the periphery of the cataract along suture lines towards the lens surface.

In the preparation of the nuclear and perinuclear regions for electron microscopic observation, it was observed that the cataractous tissue was invariably better infiltrated with plastic resin and the lens tissue less brittle than similarly prepared regions of noncataractous lenses. As a consequence, it was much easier to obtain usable thin sections of cataractous than of normal nuclear lens tissue. These differences indicated that in the selenite-induced cataract the nuclear region had become more permeable and less dense than that of the control lenses.

Discussion

The major contribution of the present study is the ultrastructural description of selenite-induced nuclear cataracts. To our knowledge, this is the first ultrastructural description of a mineral-induced cataract. The description was confined to the deep cortical and nuclear regions of early, but pronounced, selenite cataracts. A previous light microscopic study of mature...
Selenite-induced cataract revealed that the cataract had the following characteristics: atypical lens fibers, which were enlarged in the nuclear area, but decreased in the cortical region of the lens; microglobular formations and possible calcification in the nuclear area; increased vacuolization in the equatorial region under the anterior epithelium; focal hyperplasia of the anterior epithelium; cellular damage in the cortex; decreased lens diameter; and decreased anterior-posterior lens dimension. A light microscopic study of the selenite-induced cataract in rabbits showed proliferation of epithelial cells and some vacuolization. From the light microscopy of the present study, we can now add that the nuclear region of lenses showing selenite-induced cataract had a lacy or honeycombed appearance, which was due to aggregation of the cytoplasmic material.

Transmission electron microscopy further revealed the nature of the lacy or honeycomb appearance of the nuclear fibers of the cataractous lens. Unlike the dense, featureless cytoplasm normally seen in the nuclear region of control animals, the cytoplasm of nuclear fibers from rats with selenite-induced cataracts appeared to have precipitated. This precipitation of the cytoplasm may have caused the observed distortion in cell shape.

An interesting observation was the presence of a band of precipitated cytoplasm along the cell membrane except at regions of cell junctions, which made many of the cells appear continuous with one another when examined at low magnifications. Although these junctions are commonly referred to as gap junctions, Zampighi and co-workers recently have shown in the bovine lens that such junctions are both structurally and chemically different from gap junctions found in the liver. Although the function of the "gap" or "penta-laminar" junctions in the lens has yet to be determined, the absence of precipitated cytoplasm along the junction suggests that the cytoplasmic matrix simply may be attached to the cell membrane except at the junctions, or that the material may be pushed to the side of each junction by the movement of water from one cell to another.
The absence of other ultrastructural descriptions of a mineral-induced cataract makes direct comparisons with other studies impossible. In addition, although ultrastructural changes in the epithelial and cortical cells have been studied extensively in many types of cataracts, cells in the nuclear region have not been studied well. The reason for this may lie, at least in part, in the difficulty involved in preparing suitable thin sections. There are, however, several studies involving hereditary cataracts in mice, x-ray-induced cataracts, and advanced senile nuclear cataracts, all of which have shown evidence of a precipitation of the cytoplasmic proteins in nuclear fibers resulting in the formation of some large aggregates of material not unlike that we have observed in the selenite-induced cataract.

Biochemical studies of selenite-induced cataracts support the hypothesis that the electron opaque aggregates seen in the cell matrix by electron microscopy are the result of precipitation of cytoplasmic proteins.

For example, one of the most outstanding features of these cataracts is the rapid and significant increase in the amount of insoluble protein in the lens. Thus, it is likely that the lacy networks observed in the nuclear fibers and the cytoplasmic aggregations seen just inside the membrane are insoluble protein. A similar increase in insoluble material accompanied by an increase in the granularity of the cytoplasm has been reported in x-ray cataracts in rabbits.

Studies also have shown that the increase in insoluble protein seen in the selenite-induced cataract is accompanied by a dramatic loss of glutathione and NADPH before formation of the nuclear cataract. Selenite, which oxidizes the sulfhydryls of glutathione and proteins in vitro, has been postulated to cause proteins to precipitate by disulfide formation in vivo. However, if this does occur in the selenite-induced cataract, it must be confined either to a specific class of proteins or to a limited anatomical spot, since the total lens concentrations of disulfides, diglutathione, and mixed disulfides do not increase. Clear areas were observed around the aggregates of precipitated cytoplasm.
hereditary nuclear cataracts of the Nakano mouse\textsuperscript{17} the cytoplasm also clumps, forming large aggregates. This occurs only after there has been a significant increase in lens sodium concentration. The sodium increase has been shown to be the result of a Na-K ATPase deficiency. Consequently, in the selenium-in-
duced cataract water uptake could be involved since an increase in total lens water has been observed when these cataracts are hypermature. However, no changes were observed in the concentrations of sodium, potassium, or water in well-formed nuclear cataracts examined up to 12 days after a single injection of selenite. These results would suggest that hydration is a result rather than a cause of the selenite-induced cataract. However, it will be necessary to measure the water and ion levels present in cataracts produced by multiple injections of selenite before it can be determined whether or not the changes we observed were indeed due to hydration.

Key words: nuclear cataract, selenite, transmission electron microscopy, light microscopy, lens

References