Galactose cataract prevention with sorbinil, an aldose reductase inhibitor: a light microscopic study

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Cataract formation in galactosemic rats was studied by ophthalmoscopy, slit-lamp biomicroscopy, and by light microscopy using plastic embedding with methacrylate. Untreated rats developed nuclear cataracts by 14 days and mature cataracts by 21 days. However, rats treated with the aldose reductase inhibitor sorbinil did not develop any cataractous change for up to 8 months of 50% galactose feeding and could not be distinguished from normal controls. This strongly suggests that aldose reductase is the common factor involved in the formation of sugar cataracts. (INVEST OPHTHALMOL VIS SCI 22:174-179, 1982.)

Key words: galactosemic cataract, aldose reductase inhibitor, methacrylate plastic embedding

Aldose reductase (AR) has been implicated as the common factor which initiates the cataractous process in both diabetic and galactosemic rats. A variety of AR inhibitors with diverse chemical structures has been shown to effectively delay the onset of cataracts in diabetic and galactosemic animals. Moreover, the inhibitor sorbinil (d-6-fluorospirochroman 4,4'imidazolidine 2',5'dione) has recently been reported to essentially prevent cataractous changes from occurring in diabetic rats. To complete documentation on the treatment of sugar cataracts with AR inhibitors, we present a light microscopic study of the lenses of galactosemic rats treated with sorbinil.

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Methods
Male Sprague-Dawley albino rats weighing 50 gm were fed 50% galactose feed. Rats were treated with sorbinil given by oral intubation at a dose of 60 mg/day/kg body weight. Subsequent experiments showed that the same results were obtained when the sorbinil was mixed with rat feed (425 mg/kg of food). A total of 250 rats in each of the untreated and sorbinil-treated groups were used.

The eyes were examined daily with pupils dilated by a cycloplegic-mydriatic (0.5% to 1.0% cyclopentolate (Cyclogyl); Alcon Laboratories, Inc., Ft. Worth, Texas), and the lenses were examined with an American Optical direct ophthalmoscope and an American Optical slit-lamp Biomicroscope. For histology, samples were taken at 3, 7, 10, 14 and 21 days.

The rats were sacrificed with an overdose of sodium pentobarbital; the eyes were carefully enucleated and then fixed overnight in 4% glutaraldehyde in 0.15M sodium-potassium phosphate buffer, pH 7.2. The eyes were slit open at the limbus 10 min after start of fixation to allow the rapid entry of the fixative. The eyes were transferred to 10% buffered formaldehyde the next day and further fixed for several days. They were then dehydrated for 2 hr each in a series of ethyl alcohols (50%, 70%, 80%, 90%, and 95%) and
infiltrated with three changes of plastic (methacrylate) embedding mixture, solution A with catalyst (JB-4 embedding kit; Polysciences Inc.) at 2 hr intervals. A final embedding mixture, 20 to 25:1 ratios of solution A with catalyst and solution B, was poured into plastic molds. The well-infiltrated whole eyes were then dropped into the molds and positioned, and the medium allowed to harden. Within 30 to 45 min, the medium had polymerized sufficiently hard to allow an oily residue to be washed off the surface with soapy water. The molds were then left overnight to harden, and the block was cut the next day on a Sorvall JB-4A microtome. Sections 1 to 2.5 \( \mu \text{m} \) thick were cut with a ½-inch thick glass knife. The sections were floated on water, mounted on a glass slide, dried on a hot plate, and stained (hematoxylin/eosin, periodic acid–Schiff stain (PAS), or toluidine blue). They were then covered with a cover slip, examined, and photographed with a Carl Zeiss microscope.

Samples were also processed by American Histolabs (Rockville, Md.).

In the histological studies we found that the plastic-embedding method, a procedure not widely used for the lens, yielded results far superior to that of the paraffin-embedding technique. The whole eye was routinely embedded as in the paraffin procedure. This avoids introducing artifacts caused in dissecting out the fresh lens. Moreover, with the plastic method it is possible to section the eye so as to obtain sections as thin as 1 \( \mu \text{m} \). The water-insoluble plastic rapidly infiltrates and is not affected by high humidity. Furthermore, the simple procedure does not require ultraviolet irradiation. Routine staining methods used for paraffin are also applicable to the plastic fixed material.

The simple method allows for a magnification of up to 1000X with light microscopy, gives excellent definition of cataractous changes, and is relatively free of artifacts. When many cataracts are being examined, the use of the relatively thin section of plastic-embedded specimens is the procedure of choice. When greater definitions of a particular area of the cataract is required, electron microscopy can be employed.

Results

**Ophthalmoscopic and slit lamp observations.** Cataracts in the galactose-fed rats progressed almost at the same rate in all animals, and the dense nuclear opacity formed usually 14 days after the start of the diet. The first obvious change were vacuoles at the equator at the third day on the galactose diet. All rats developed a nuclear opacity that was grossly visible by 2 weeks and had mature cataracts by day 21 (Fig. 1). In striking contrast, all rats treated with sorbinil showed no lens changes when examined by the ophthalmoscope or slit lamp Biomicroscope although they had been maintained on the 50% galactose diet for up to 8 months. The sorbinil-treated rats were examined daily for the first month on the galactose diet and weekly thereafter. These lenses could not be distinguished from the normal control rat lenses. Sorbinil effectively blocked the synthesis of galactitol, so that the level of polyol accumulation in the lenses were less than 10% of those in the untreated galactosemic rats.

**Histological changes of the lenses**

**Untreated, galactose-fed rats**

**Initial vacuolar stage.** Three days after initiation of the 50% galactose feed, the lenses of all rats showed pre-equatorial and equatorial vacuoles (Fig. 2). These were described previously as located between fibers and were called intercellular cysts.\(^7\) The lens fibers were swollen, and those in the bow area appeared particularly vulnerable.

**Intermediate vacuolar stage.** Seven days after the start of the galactose diet, the swell-
Figs. 2 to 6. For legends see facing page.
ing of the lens fibers and formation of vacuoles were prominent in the anterior and posterior cortical area. In some areas, there was already liquefaction of cortical lens fibers. Abnormal local proliferation of anterior lens epithelial cells was also observed (Fig. 3). These abnormally proliferating cells continued to produce basement membrane in a disorderly fashion.

**LATE VACUOLAR STAGE.** There was further liquefaction of the cortical lens fibers as well as proliferation of anterior epithelial cells at 10 days. The anterior and posterior Y sutures were seen with gaping spaces, and the gaps were filled with what appeared to be liquefied cortical material. The supranuclear lens fibers were swollen, and the ends of the fibers appeared to have swollen many times the normal size (Fig. 4). The cells in the bow area were also edematous.

**NUCLEAR CATARACT STAGE.** At 14 days, the cortex was mainly liquefied. The bow area still showed actual formation of new lens fiber cells. Extensive liquefaction occurred in the area of the Y sutures. The lens epithelial cells showed foci of proliferation and evidence of multilayering of cells. The lens nucleus was noted to be floating in the fluid cortex.

**MATURE CATARACT STAGE.** At 21 days, the cortex, as already seen on day 14, was liquefied (Fig. 5). The bow area still showed formation of new lens fiber cells, although the new fiber cells were markedly swollen. The epithelial cells continued to proliferate and form multilayers of cells. The nucleus was displaced. The deep lens fibers were disintegrating.

**Sorbinil-treated galactose-fed rats.** Close histological examinations of the lenses of the sorbinil-treated rats fed galactose for 3, 7, 10, 14, and 21 days revealed no change, and such lenses could not be distinguished from normal control rat lenses. Changes seen in the untreated group could not be detected in the treated group.

Similarly, histological examination of the lenses of rats fed galactose and treated with sorbinil for 1, 3, 6, and 8 months revealed no cataractous changes, and such lenses could not be distinguished from normal control rat lenses (Fig. 6).

**Discussion**

Cataracts that can be induced in galactosemic rats occur much more readily than in diabetic rats. Early lens changes detectable in lenses of rats fed galactose for only 3 days require 2 to 3 weeks to occur in diabetic rats. A dense nuclear opacity in galactose-fed rats occurs in 2 weeks, whereas in diabetic rats it appears in 9 to 12 weeks.8–12

Sugar level obviously is an important factor. However, the difference in the rate of cataract formation between the galactose and diabetic rats is not related to the sugar levels. In fact, the blood galactose level in the galactosemic rats is usually one half or less of the

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**Fig. 2.** Initial vacuolar stage. Bow area of the lens shows vacuoles forming in the pre-equatorial and equatorial cortex. (Methacrylate, toluidine blue; ×200.)

**Fig. 3.** Intermediate vacuolar stage. Anterior subcapsular area shows abnormal proliferation of cells with formation of basement membrane in a disorderly fashion (arrows). Area of the anterior suture is filled with liquefied cortical material (asterisk). Note the swollen ends of the cortical lens fibers. (Methacrylate, toluidine blue; ×200.)

**Fig. 4.** Late vacuolar stage. Cross section of the lens fibers in the posterior supranuclear area shows swollen fibers many times the normal size. (Methacrylate, toluidine blue; ×200.)

**Fig. 5.** Mature cataract stage. Bow area of the lens still shows formation of new lens fibers despite the complete liquefaction of the cortex. The newly formed fibers are edematous. (Methacrylate, toluidine blue; ×200.)

**Fig. 6.** Sorbinil-treated galactosemic rat, after 8 months on 50% galactose diet. Section of bow area shows none of the changes seen in the untreated rats and cannot be distinguished from the normal controls. (Methacrylate, toluidine blue; ×200.)
glucose level in diabetic rats. The difference is explained by the fact that galactose is a better substrate for AR than glucose. Second, sorbitol is further metabolized but galactitol is not. As a result of these two factors, the osmotically active products of the sorbitol pathway never accumulate as highly in the lens of a diabetic rat as in the galactosemic rat.

In sugar cataract formation, age is another factor affecting the rate of opacification with cataract more easily induced in the younger animal. It is therefore most impressive that sorbinil is so effective in preventing cataractous changes from occurring in young weanling rats fed a 50% galactose diet. The histological studies revealed that there was no evidence of precataractous changes during the 8 months of observation.

A summary of the various studies of AR inhibitors on sugar cataract formation reveal the striking effectiveness of sorbinil (Tables I and II). The sorbinil dose of 60 mg/kg/day used in the present study was much lower than that of AR inhibitors in other studies. For example, alrestatin was used at 700 to 1100 mg/kg/day and quercitrin at 3000 mg/kg/day. In all likelihood a lower level of sorbinil may be effective, since the 60 mg dose was the only level used. However, Peterson et al., who first introduced sorbinil, reported that a 10 mg/kg/day dose was not effective in completely preventing lens changes. The most severe test of any AR inhibitor is to block cataracts when a 50% galactose diet is fed to young rats. In some studies in Table I a lower cataractogenic concentration of galactose was used to demonstrate the effectiveness of the AR inhibitor. Thus far, sorbinil at a sufficient dose is the only inhibitor to successfully prevent cataracts under these conditions.

### Table I. Galactose cataracts in rats

<table>
<thead>
<tr>
<th>% galactose in diet</th>
<th>AR inhibitor</th>
<th>Route of administration</th>
<th>Effect on polyol level (%)</th>
<th>End point of cataract</th>
<th>Cataract development</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>Alrestatin</td>
<td>Oral</td>
<td>50</td>
<td>Nuclear opacity</td>
<td>Delay, 3 weeks</td>
</tr>
<tr>
<td>50</td>
<td>Alrestatin</td>
<td>Oral</td>
<td>50</td>
<td>Nuclear opacity</td>
<td>Delay</td>
</tr>
<tr>
<td>50</td>
<td>Ay 20,263</td>
<td>Vitreous injection</td>
<td>50</td>
<td>Vacuoles and nuclear opacity</td>
<td>Delay, 2 weeks</td>
</tr>
<tr>
<td>50</td>
<td>Quercitrin</td>
<td>Topical</td>
<td>50</td>
<td>Nuclear opacity</td>
<td>Delay, 1 week</td>
</tr>
<tr>
<td>30</td>
<td>Sorbinil</td>
<td>Oral</td>
<td>96</td>
<td>Vacuoles</td>
<td>Marked delay</td>
</tr>
<tr>
<td>35</td>
<td>R.S. 7535</td>
<td>Topical and oral</td>
<td>—</td>
<td>Nuclear opacity</td>
<td>Delay</td>
</tr>
<tr>
<td>50</td>
<td>Quercitrin</td>
<td>Oral</td>
<td>50</td>
<td>Nuclear opacity</td>
<td>Delay</td>
</tr>
<tr>
<td>50</td>
<td>Quercitrin</td>
<td>Oral</td>
<td>50</td>
<td>Nuclear opacity</td>
<td>Delay</td>
</tr>
<tr>
<td>50</td>
<td>Sorbinil</td>
<td>Oral</td>
<td>90</td>
<td>Vacuoles and nuclear opacity</td>
<td>No lens changes in 8 mo</td>
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### Table II. Diabetic cataracts

<table>
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<tr>
<th>Animal</th>
<th>AR inhibitor</th>
<th>Route of administration</th>
<th>Effect on polyol level</th>
<th>End point of cataract</th>
<th>Cataract development</th>
<th>Investigator</th>
<th>Ref. No.</th>
<th>Year</th>
</tr>
</thead>
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<tr>
<td>Degu</td>
<td>Quercitrin</td>
<td>Oral</td>
<td>50%</td>
<td>Vacuoles and nuclear opacity</td>
<td>Delay</td>
<td>Varma et al.</td>
<td>3</td>
<td>1977</td>
</tr>
<tr>
<td>Degu</td>
<td>Quercitrin</td>
<td>Oral</td>
<td>50%</td>
<td>Vacuoles and nuclear opacity</td>
<td>Delay 2 weeks</td>
<td>Varma</td>
<td>16</td>
<td>1978</td>
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<tr>
<td>Rat</td>
<td>Sorbinil</td>
<td>Oral</td>
<td>95%</td>
<td>Vacuoles and nuclear opacity</td>
<td>No lens changes in 6 mo</td>
<td>Fukushi et al.</td>
<td>6</td>
<td>1980</td>
</tr>
<tr>
<td>Degu</td>
<td>Sorbinil</td>
<td>Oral</td>
<td>95%</td>
<td>Vacuoles and nuclear opacity</td>
<td>No lens changes in 6 mo</td>
<td>Datiles and Fukui</td>
<td>Unpub data</td>
<td>1980</td>
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Animals were made diabetic with streptozotocin injections.
The fact that so many structurally different AR inhibitors are effective is strong support for the concept that AR is involved in the formation of sugar cataracts. The fact that these inhibitors are consistent in delaying the formation of cataracts in both diabetic and galactosemic rats is further proof that AR is the common factor involved in the initiation of these cataracts.

REFERENCES