Reversal of Galactose Cataract with Sorbinil in Rats

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Sorbinil, a potent aldose reductase inhibitor, can effectively block the progression of a galactose cataract even though the cataractous process is well underway. The prevention of dulcitol accumulation by Sorbinil is just as effective in reversing the cataract as the removal of galactose from the diet. The progression and reversal of the cataract were followed by ophthalmoscopy and histology. The results also further support the concept that in galactosemia the cataract is not caused by the toxic effects of galactose per se but by the consequence of the aldose reductase reaction. Invest Ophthalmol Vis Sci 24:640–644, 1983

Aldose reductase is implicated as a factor initiating the cataractogenic process in sugar cataracts. In the presence of high sugar levels, the corresponding sugar alcohol is accumulated in the lens fiber cells through the polyol pathway of sugar metabolism. This results in an increase of osmotic pressure that in turn exerts an osmotic stress on the lens fibers. These specific biochemical changes are consistent with early histopathologic changes of the lens of galactosemic rats. The pathology is characterized by a swelling of lens fibers in the equatorial region. Extensive biochemical and histopathologic studies on the experimental galactose cataract have shown that the cataractous process in galactosemic animals can be reversed if the diet containing galactose is replaced by a normal diet at a critical time. Datiles et al have demonstrated that the rats treated with the aldose inhibitor, Sorbinil (S-6-fluoro-spiro-chroman-4, 4′-imidazolidine 2′,5′ dione) did not develop any cataractous changes in the lenses for up to 8 months of 50% galactose feeding. In this study we examine the question of whether Sorbinil can effectively block the progression of a galactose cataract once the process is well established, and we compare the effectiveness of Sorbinil treatment to the reversal of the cataract achieved by removal of galactose from the diet.

Materials and Methods

Fifty Sprague-Dawley rats, 3 weeks old, weighing about 50 g, were fed a 50% galactose diet (United States Biochemical Corporation, Cleveland, OH) for 5 days. After this period on the galactose diet, the rats were divided in three groups: (1) maintained on the galactose diet throughout the experiment (18 rats); (2) galactose diet replaced with the normal diet (14 rats); (3) galactose diet replaced with the Sorbinil-galactose diet (400 mg Sorbinil mixed with 1 kg of 50% galactose diet [16 rats]). The three groups were kept on this regimen for varying periods up to 2 months. The pupils were diluted with 1% cyclogyl, and the lenses were examined by retroillumination with the direct ophthalmoscope at different periods. The rats were killed at varying periods of time up to 2 months. Lenses were dissected and homogenized in 0.3 N zinc sulfate, 1 ml/lens after which the homogenate was neutralized by an equivalent amount of 0.3 N barium hydroxide and centrifuged at 10,000 × g for 20 min. A 1-ml aliquot of supernatant was lyophilized overnight, and the lenticular dulcitol levels were measured by the gas liquid chromatographic method as previously described. For histopathologic study of rat lenses, the eyes were enucleated, and the lenses were prepared as described by Datiles et al. Nine additional galactosemic rats had their diet replaced with Sorbinil-galactose on day 7 and were followed up by ophthalmoscopic examination.

Results

Clinical Feature

Examination of the galactosemic rat lenses through dilated pupils revealed that some peripheral opacities appeared as early as day 3. At day 5 the formation of a complete ring of opacities at the equatorial region was striking. For the rats fed galactose throughout the experiment, the opacities increased with time and progressed to a dense nuclear opacity by 2 weeks and complete opacification by 3 weeks. The other two groups of rats, either on a galactose diet replaced by
a normal diet or on the Sorbinil-galactose diet, had almost the same, if not identical, gross changes of the lens throughout the experiment. At day 20 (15 days after reversal), the equatorial opacities became fragmentary, changing to an incomplete ring of tiny opacities that further decreased with time. By day 40–45, the lenticular opacities of all rats but one disappeared, with the lenses becoming grossly clear. One rat fed with the Sorbinil-galactose diet had an incomplete ring of equatorial opacities at day 20 that remained unchanged up to day 60. However, the lenticular dulcitol levels of this particular rat was found to be definitely higher than those of the other rats in the same group (9.7 vs 1.8 μmoles/gm for the others).

It appears that complete reversal of the cataract is not achieved if the rats are maintained on the 50% galactose diet beyond 5 days. When the reversal is begun either with the normal diet or Sorbinil treatment after 6 days on the galactose diet, the process progresses to the nuclear cataract stage within 4 weeks. In this case the cortex appears to clarify but the nuclear opacity develops and persists. These results are consistent with what was observed before.

Lenticular Dulcitol Level

The lenticular dulcitol levels of the rats are shown in Figure 1. At day 5 the dulcitol level of the galactosemic rats was found to be 73 μmoles/g, which is consistent with previously reported results. This increased further to a peak of 93.5 μmoles/g on day 10, just prior to the appearance of a dense nuclear opacity. As noted previously the dulcitol levels then decreased dramatically at day 15 and remained around 25 μmoles/g after the subsequent appearance of the nuclear opacity. In the case of the reversal group, rats fed either with a normal diet or the Sorbinil-galactose diet on day 6, the lenticular dulcitol levels followed almost identical patterns of change up to day 60. The dulcitol levels of these rats fell rapidly during the first few days after reversal, by day 10 it had decreased to 22–28 μmoles/g, and by day 20 onward it had further decreased approaching zero. These results suggest that the level of lenticular dulcitol after replacement with the Sorbinil-galactose diet or a normal diet no longer exerts any significant osmotic stress on those rat lenses.
Histopathologic Changes of the Lens

The histopathologic lens changes of rats fed with galactose throughout the experiment were the same as those described previously in this laboratory. Figure 2 shows that after 5 days of galactose there is an extensive area of disintegration of fibers forming vacuoles in the pre-equatorial and equatorial cortex in the bow region. In the galactosemic rats at day 10 histology revealed a dramatic swelling of the supranuclear lens fibers and marked liquefaction of the cortical fibers (Fig. 3). On the other hand, at day 10 of the Sorbinil-treated galactosemic rats or those switched to a normal diet, the histopathologic lens picture remained almost unchanged from those found in the initial vacuolar stage. There was a confluence of the vacuoles at the anterior cortex, mild swelling of the lens fibers, and evidence of new fiber formation at the bow area (Fig. 4). After an additional 5 days of Sorbinil treatment, day 15, areas of large vacuoles began to disappear, presumably because newly formed fibers from the epithelial cells eventually filled the defective region. As shown in Figure 5, by day 60 the histology of the lens from the rats transferred to a
normal diet or the Sorbinil-galactose diet appeared normal, indicating that in both cases complete reversal of the cataract had occurred.

**Discussion**

It is established that aldose reductase is responsible for triggering the mechanism of sugar cataracts in rats. Aldose reductase converts sugar to polyol, which leads to its accumulation and results in a hypertonicity within the lens fibers. The osmotic change eventually results in a disruption of lens fibers.11,12 Associated with the break down of the membrane barrier is the reversal of intracellular Na/K ratio,5,11 a decrease in lenticular glutathione,5 and free amino acids,5,11 as well as a decrease in crystallin synthesis.7 At the dense nuclear cataract stage, permeability is compromised so that a dramatic decrease in dulcitol content occurs.13,14 At this stage only the large lens proteins are retained in the lens. The result is a large influx of sodium and chloride ions and water, typical of a Donnan type of swelling.1

With the availability of aldose reductase inhibitors, it is now possible to block effectively the formation of sugar cataracts in rats. Peterson et al. first introduced the inhibitor Sorbinil, and found it to retard cataract development in the galactosemic rat at oral dosages as low as 5 mg/kg/day.15 At higher dosages Sorbinil was found to prevent successfully cataracts in both the diabetic and the galactosemic rats.8,16 In this study we explored the effectiveness of Sorbinil in checking the progress of a cataract already underway. We have presented evidence that Sorbinil treatment can reverse the pathologic process in rats fed galactose for 5 days just as effectively as if the galactose were withdrawn from the diet. In both cases the vacuoles disappeared and the nuclear opacity never developed. If the galactose feeding is extended beyond 5 days, however, the cataract reversal is not complete. For example, a 6-day feeding of the galactose diet followed by Sorbinil treatment or switching to a normal diet did not prevent the nuclear opacity from occurring. These observations on cataract reversal were made on rats fed a 50% galactose diet. Obviously if a lower concentration of galactose were used, successful reversal may occur at a later period than day 6. Another factor is the age of the animal. A longer time period is required for a cataract to develop in older rats fed a 50% galactose diet. Presumably the time frame for cataract reversal would also be different if older rats were used.

During the reversal phase of experimental galactose cataract with normal diet, it has been demonstrated previously that the lenticular glutathione and free amino acids return to near normal levels, and that a partial recovery of lenticular potassium5 and crystallin synthesis also occurs.7 Since the replacement with the Sorbinil-galactose diet proved to abolish effectively the polyol pathway of sugar metabolism, as evidenced by a progressive decrease in the lenticular dulcitol level, all of the above mentioned changes might also occur in the reversal phase of these galactosemic rats. This in turn should lead to the re-establishment of normal lens physiology. Thus, it is not unexpected that the gross appearance of the lens dulcitol levels and histologic changes of the cataract
reversal in the galactosemic rats treated with Sorbinil appeared identical to those found in rats fed a normal diet from day 6. These findings observed in rat studies suggest that Sorbinil may have beneficial effects in the treatment of human galactosemic and diabetic cataracts.

Key words: lens, galactose cataract, sorbinil

References