The Effects of Sorbinil, an Aldose Reductase Inhibitor, on the Corneal Endothelium in Galactosemic Dogs

Manuel D. Datiles, Peter F. Kador, Kayoko Kashima, Jin H. Kinoshira, and Anvira Sinha

Wide-field specular microscopy was used to examine the central corneal endothelium of age- and sex-matched beagle dogs fed for up to 32 months either normal control diets containing 30% nonnutrient filler (13 dogs) or diets containing 30% galactose with (13 dogs) or without (12 dogs) concomitant treatment with the aldose reductase inhibitor, sorbinil. Computerized morphometric analysis of the endothelial cells indicated that a significant decrease in cell density and increase in mean cell area occurred in untreated galactose-fed dogs after 32 months of feeding compared with the normal controls. However, no significant difference could be observed in similar galactose-fed dogs treated with sorbinil. No significant difference in the coefficient of variation of the area, or percent hexagonality of the endothelial cells, or the corneal thickness could be observed in any group. These findings demonstrated that endothelial abnormalities were present in the cornea of the galactose-fed dogs which were similar to those reported for diabetic dogs, rats, and patients and that these changes can be prevented by the concomitant administration of an aldose reductase inhibitor. These findings suggest a role for aldose reductase in the abnormalities noted in the corneal endothelium in diabetes and galactosemia.

Diabetes-associated corneal complications include nonhealing and recurrent epithelial defects in patients,1,2 epithelial healing abnormalities in animals,3-6 and abnormalities in the corneal endothelium.7-9 Corneal endothelial abnormalities include changes in the appearance of endothelial cells with polymegathism (high coefficient of variation in cell area) and pleomorphism (marked decrease in the percentage of hexagonal cells). These changes were reported in diabetic patients compared with age-matched controls,7 in diabetic rats,8,9 and diabetic dogs.10

Aldose reductase was implicated in corneal epithelial complications, and the presence of this enzyme in the corneal epithelium was demonstrated by immunohistochemical localization.11 In diabetic and galactosemic rats, aldose reductase inhibitors were effective in correcting abnormalities in corneal reepithelialization.3-6 Moreover, in several clinical cases aldose reductase inhibitors were able to promote healing of sight-threatening epithelial defects in diabetic patients who underwent vitrectomy or laser photocoagulation.6,12 Aldose reductase was also demonstrated to be present in the corneal endothelium,11 and recently, it was shown that aldose reductase inhibitors can prevent corneal endothelial abnormalities in diabetic rats.8,9 If aldose reductase is involved in these corneal endothelial abnormalities, similar changes should also occur with galactosemia. To examine this possibility we investigated corneal endothelial changes in age- and sex-matched galactose-fed dogs and compared the results with those obtained in similar galactose-fed dogs concomitantly treated with the aldose reductase inhibitor, sorbinil, and in dogs fed a normal control diet.

Materials and Methods

The studies involving animals conformed to the ARVO Resolution on the Use of Animals in Research.

Nine-month-old male beagle dogs (Marshall Farms USA, North Rose, NY) were individually housed in 3x9-ft runs. After slit-lamp and funduscopic examinations, the dogs were randomly divided into groups, with each dog receiving a daily diet (approximately 450 g) of dog chow containing either 30% nonnutrient filler (control diet) (13 dogs) or 30% galactose filler (treatment diet) (13 dogs).
(galactose diet) (25 dogs). Both diets were prepared as color-coded pellets (Bioserve, Frenchtown, NJ). One group of galactose-fed dogs served as the untreated group (12 dogs), and the other group (13 dogs) received the aldose reductase inhibitor, sorbinil (S-6-fluoro-spinocrom-an-4,5-imidazolidine-2',4'-dione; Pfizer, Groton, CT). Sorbinil tablets were initially administered at a single daily dose of 625 mg 1 hr before feeding. After 4 months the total weekday dose of sorbinil was increased to 875 mg administered as follows: 250 mg 1 hr before feeding, 250 mg 1 hr after feeding, and 375 mg approximately 8 hr after feeding. Table 1 shows blood levels of sorbinil. Twenty-four months after the start of the experiment, the corneal diameters of each of the dogs was measured using a surgical caliper. The central corneal thickness was measured three times using a DGH2001 ultrasonic pachymeter (DGH Technology, Frazer, PA), and an average was determined. Thirty-one months after the start of the experiment, the dogs were examined under anesthesia (intravenous Rompun (Mobay Corp., Shawnee, KA) and Ketalar (Parke-Davis, Morris Plains, NJ)). The central cornea of one eye from each dog was photographed using a wide-field specular microscope (Keeler Konan 40X, Keeler Instruments, Broomall, PA) (Fig. 1). In most cases, one eye had already been enucleated for another study. In those dogs with two eyes, one eye was randomly chosen for photography. Approximate ten to 15 photographs were taken of each cornea, and the best frame was selected for each eye. The magnification was calibrated using a special contact lens with grids obtained from Biometrics, Boston, MA. In each micrograph 100 adjacent cells were identified and digitized into an International Business Machines (White Plains, NY) PC computer using a SAC digitizing tablet and a graphics tablet pen (Bio-Optics Cell Endothelial Analysis System, Bio-Optics, Inc., Arlington, MA). The coordinates of individual cell apices were digitized by touching the apices or corners with the graphics tablet pen. The number of apices of each cell permitted quantitation of the percentage of hexagonal cells present which was used as a measure of cell shape variability (pleomorphism). Endothelial cell density or cells per mm² was calculated by dividing 10⁶ by the mean cell area in μm². The coefficient of variation in cell area (CV) was calculated by dividing the standard deviation of the cell area by the mean cell area. This index provides an objective measurement of cell variability (polymegethism) which is independent of cell area or cell density. Statistical analyses were done on the Prophet computer system (Prophet, Co. Inc., Cambridge, MA) using the Kruskal-Wallis and Dunn test for significance.

**Results**

Table 2 shows the corneal diameters and corneal thicknesses measured. There were no significant differences in diameter and thickness among the three different groups.

Figure 1 shows representative endothelial specular micrographs from each of the three groups studied:

### Table 1. Blood levels of Sorbinil (μg/ml)

<table>
<thead>
<tr>
<th>Weekday</th>
<th>Weekend</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr (1 hr prior to AM feeding)</td>
<td>17.7 ± 5.8</td>
</tr>
<tr>
<td>2 hr</td>
<td>25.4 ± 6.8</td>
</tr>
<tr>
<td>4 hr</td>
<td>29.7 ± 6.9</td>
</tr>
<tr>
<td>8 hr</td>
<td>19.9 ± 5.8</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

### Table 2. Corneal diameter and thickness

<table>
<thead>
<tr>
<th></th>
<th>Corneal thickness (μm)</th>
<th>Corneal diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 13)</td>
<td>609 ± 23</td>
<td>16.7 ± 0.4</td>
</tr>
<tr>
<td>Galactosemic (n = 12)</td>
<td>583 ± 81</td>
<td>16.7 ± 0.4</td>
</tr>
<tr>
<td>Galactosemic + ARI (n = 13)</td>
<td>558 ± 51</td>
<td>16.4 ± 0.3</td>
</tr>
</tbody>
</table>

Values are mean ± SD.
normal control, galactosemic, and sorbinil-treated galactosemic dogs. Table 3 shows the results of endothelial cell computerized morphologic analysis. The untreated galactosemic dogs had significantly larger cells \( (P < 0.01) \) and a significantly lower cell density \( (P < 0.01) \) compared with normal control dogs. However, when the sorbinil-treated galactosemic dogs were compared with controls, there was no significant difference in cell area or density. Comparing the CV and percent hexagonality among the three groups, no significant difference was seen.

### Discussion

A previous study in diabetic rats by Meyer et al\(^9\) showed corneal endothelial morphometric changes that topical aldose reductase inhibitors were able to prevent and reverse. Our study demonstrates that galactosemic dogs can also develop corneal endothelial abnormalities which can be prevented by an aldose reductase inhibitor, sorbinil. This finding supports the role of aldose reductase in this corneal abnormality. Previous reports indicated that the diabetic cornea is less capable of recovering from stress, as evidenced by the development of stromal edema in postvitrectomy patients with recurrent erosions and punctate keratopathy.\(^1,2\) In diabetic and galactosemic rats, the corneas in untreated rats remained cloudy even after reepithelialization, while the corneas in aldose reductase inhibitor-treated diabetic and galactosemic rats did not.\(^3,5\) This persistent corneal edema may be due to poor endothelial function resulting from delayed recovery from the stress of limbus-to-limbus corneal scraping. Hence, the abnormalities observed in the corneal endothelium may reflect lower endothelial reserve, making the cornea more susceptible to injury during stress.

Diabetic dogs\(^10\) were reported to show increases in polymegathism and pleomorphism of corneal endothelial cells which corresponded directly to the level of glycemic control. However, no significant difference in cell density or area was observed, suggesting that these two measurements may not detect early corneal endothelial changes. On the other hand, in our long-term study of galactosemic dogs, abnormalities in cell density and area were observed; however, no significant changes in CV and percent hexagonality could be detected. This suggests that although cells may be damaged and die, the remaining surviving cells may be able to recover and regain normal shape in the long run. The galactose diet did not affect the growth of the cornea since no difference in the diameter of the corneas could be detected after 2 yr of galactose feeding when the dogs reached adulthood.

Although the exact mechanism is unclear, it is possible that aldose reductase causes polyol accumulation in the endothelial cell which may then injure the cell and prevent its proper metabolic functioning. These changes may be corrected by use of aldose reductase inhibitors which reduce polyol production. Therefore, it may be beneficial to treat patients with aldose reductase inhibitors before they undergo stress such as a vitrectomy, laser photocoagulation, or similar procedures to prevent the occurrence of corneal problems.

A study by Gwin et al\(^14\) on age-related change in endothelial cell density showed that young dogs had endothelial counts of 2600 cells/mm\(^2\) which decreased to 2300–2500 cells/mm\(^2\) between ages 1–9 yr. A similar mean value of 2621 cell/mm\(^2\) were observed in our normal control dogs, at 32 months, although our study dogs were all uniformly bred male beagles of the same age. Gwin et al's study used a mixture of beagles, Schnauzers, and dogs of various breeds and ages that underwent cataract surgery.

### Key words:
aldose reductase, corneal endothelium, diabetes, galactosemia, dog

### References


### Table 3. Endothelial cell features

<table>
<thead>
<tr>
<th></th>
<th>Cell density (cells/mm(^2))</th>
<th>Mean cell area ((\mu)m(^2))</th>
<th>Area CV</th>
<th>% Hexagonality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 13)</td>
<td>2635 ± 129*</td>
<td>385 ± 22*</td>
<td>0.19 ± 0.02</td>
<td>73 ± 7</td>
</tr>
<tr>
<td>Galactosemic (n = 12)</td>
<td>2429 ± 141*</td>
<td>413 ± 24*</td>
<td>0.21 ± 0.03</td>
<td>72 ± 7</td>
</tr>
<tr>
<td>Galactosemic + ARI (n = 13)</td>
<td>2556 ± 135</td>
<td>392 ± 20</td>
<td>0.20 ± 0.02</td>
<td>72 ± 7</td>
</tr>
</tbody>
</table>

Values are mean ± SD. Endothelial cell features were obtained by computer analysis. Only one eye per dog was studied.

* Statistically significant difference \( (P < 0.01) \).
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CORRECTION

In the article “Reduction of Basement Membrane Thickening in Diabetic Cat Retina by Sulindac,” by Sherif
Z. Mansour, et al, which appeared in the March 1990 issue of Investigative Ophthalmology and Visual Science,
the data presented was inadvertently printed as mean ± SEM when the values are actually expressed as mean ± SD. The following corrections should be made:

Page 457, Abstract, line 7: Mean ± SD instead of SEM.
Page 458, line 6 and Table 1: Mean ± SD instead of SEM.
Page 460, First paragraph of results: Mean ± SD; P < .004 instead of mean ± SEM; P < .04.
Page 461, Table 2: Mean ± SD instead of SEM.

The publisher regrets any inconvenience this error may have caused.