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Progression of Sugar Cataract in the Dog

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Young beagle dogs were fed a 30% galactose diet, with or without the aldose reductase inhibitors sorbinil or M79175. Cataract formation was monitored by indirect ophthalmoscope and hand-held slit-lamp microscopy and documented by retroillumination photography. In these dogs, the first sign of cataract development was an accentuation of the anterior and posterior lens sutures (1 month after feeding), then the appearance of cortical vacuoles (3 months after feeding), and finally, the formation of predominantly equatorial cortical opacities toward the posterior cortices (4–6 months after feeding). After long-term galactose feeding, a progressive, irregular, clear zone formed at the cortical equatorial regions. Light microscopic examination of these lenses shows that the cataracts are osmotic, many of the lens fibers appear to be swollen or ruptured, and vacuoles are seen near the bow region. Moreover, these histologic changes were reduced in a dose-dependent manner in galactose-fed dogs concomitantly treated with the aldose reductase inhibitors sorbinil or M79175. The osmotic nature of these cataracts and the observation that their formation can be reduced in a dose-dependent manner by aldose reductase inhibitors are consistent with the concept that the aldose-reductase catalyzed formation of polar sugar alcohols (polyols) initiates sugar cataract formation in the dog. Invest Ophthalmol Vis Sci 32:1925–1931, 1991

An increased incidence of cataract has been seen among diabetic and young galactosemic patients. Animal studies, conducted primarily in rodents, show that sugar cataracts, which are associated with diabetes and galactosemia, occur by a common osmotic mechanism. This mechanism is linked to the excess presence of the lenticular sugar alcohols sorbitol and galactitol.1,2 The intracellular accumulation of these polyols has produced a hyperosmotic effect that causes cellular swelling. This swelling is accompanied by an alteration of lenticular electrolyte levels, increased membrane permeability, and the formation of water clefs and vacuoles that cause reversible cortical opacities. As swelling progresses, other lenticular parameters are altered, and an irreversible nuclear cataract is eventually formed. These polyols result from the aldose reductase-catalyzed reduction of glucose and galactose3,4; moreover, the onset and progression of sugar cataracts are directly proportional to the level of aldose reductase activity in the lens.1,5 For example, sugar cataracts form rapidly under mild, glycemic conditions in the octodon degus, an animal whose lenses have three-fold greater amounts of aldose reductase activity than that of rats, whereas no cataracts form in the hyperglycemic mouse, an animal whose lenses contain low levels of aldose reductase.

Although the aldose reductase-initiated production of polyols initiates sugar cataract formation through osmotic effects, it is unclear whether a similar mechanism occurs in humans because the activity of aldose reductase in the human lens is lower than that in the rat, and the progression of cataract formation differs between humans and rats. In the rat lens, opacities form predominantly in the anterior regions,4,5 whereas in humans, posterior subcapsular opacities are formed. We report on sugar cataract formation in the dog, an animal model whose lenses contain levels of aldose reductase activity more similar to those of humans than rats.

Materials and Methods

Estimation of Aldose Reductase Activity in Rat, Dog, and Human Lenses

Frozen Sprague-Dawley rat eyes were obtained commercially (Biotrol, Indianapolis, IN). Eyes were obtained from dogs, immediately after death, and were stored at −70°C until used. Both rat and dog lenses were removed by the posterior approach from the thawed eyes before evaluation. Human lenses from patients with cataracts were frozen immediately after intracapsular cataract extraction and were stored at −70°C.

The thawed lenses were homogenized in ground glass homogenizers with Na, K-phosphate buffer, pH 7.5 containing 0.5 mM ethylenediaminetetraacetic acid

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acid (EDTA) and 10 mM 2-mercaptoethanol and centrifuged at 10,000 x g for 15 min. Aldose reductase activity in the supernatants was assayed spectrophotometrically on a Guilford Response spectrophotometer (Gilford Instrument Labs, Oberlin, OH) by following the decrease of NADPH at 340 nm using 10 mM DL-glyceraldehyde as substrate, as described above.6 One unit of activity was defined as the activity consuming or producing 1 nmole of NADPH per min at 22°C. Protein concentrations were determined according to the method of Bradford, using bovine serum albumin as a standard.7

Dogs

Nine-month-old male beagle dogs (Marshall Farms USA, Inc., North Rose, NY), were randomly divided into five equal groups, individually housed in 3 x 9 foot runs, and fed a daily diet (Bioserve, Frenchtown, NJ) consisting of about 450 g of standard dog chow containing either 30% nonnutrient filler (control diet) or 30% galactose (galactose diet). One group of galactose-fed dogs served as the untreated group, whereas the other groups received either the aldose reductase inhibitor sorbinil (S-6-fluoro-spirochroman-4,5'-imidazolidine-2',4'-dione), or M79175 (2-methyl-6-fluoro-spirochroman-4,5'-imidazolidine-2',4'-dione) as described below.8,9 In the sorbinil-treated group, sorbinil tablets (250 mg and 125 mg) were initially administered at a single daily dose of 625 mg 1 hr before feeding. After 4 months, the total weekly dosage of sorbinil was increased to 875 mg, administered as 250 mg 1 hr before feeding, 250 mg 1 hr after feeding and 375 mg approximately 8 hr after feeding. On weekends, each dog received 1250 mg administered as 625 mg 1 hr before feeding, and 625 mg approximately 8 hr after feeding. For the low-dose M79175 group, M79175 was initially administered at an average group dose of 0.5 mg/kg 1 hr before feeding. After 5 months, the weekday dosage was doubled to 1.0 mg/kg with one half administered 1 hr before and the other half approximately 8 hr after feeding. On weekends, low-dose M79175 was administered as a single dose of 0.5 mg/kg 1 hr before feeding. For the high-dose M79175 group, M79175 was administered on weekdays at an average group dose of 5.0 mg/kg administered as three equal doses 1 hr before feeding, 1 hr after feeding, and approximately 8 hr after feeding. On weekends, 4.0 mg of M79175 was administered as two equal doses 1 hr before and 8 hr after feeding. Experiments on all dogs conformed to the ARVO Resolution on the Use of Animals in Research.

Ophthalmic Examination of Cataracts

Dogs were periodically examined with the indirect ophthalmoscope and hand-held slit lamp, preceded by mydriasis with topical administration of 1% tropicamide hydrochloride. Severity of cataracts was subjectively classified on a scale of 0–4, with 4 being the most progressive type of cataract. The classifications were as follows: 1) an incipient cataract with some degree of vacuolization in the anterior or posterior cortex; 2) a progression of vacuoles and opacification to stria in the cortex with involvement of the sutures; 3) opacification of most of the visible cortex; and 4) a complete dense cataract.

Retroillumination Photography

The eyes of nonanesthetized dogs were dilated with 1% mydriacyl and 10% neosynephrine and restrained by hand in a suspended standard animal sling. Photographs were taken with a Neitz CT-R retroillumination camera (Nertz Instrument Co., Ltd., Tokyo, Japan). Because of size differences between the dog and human eye, composite photographs were constructed to obtain photographs of the entire dog lens.

Histologic Features

Lenses were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, and dehydrated with subsequent washes of 50%, 70%, 85%, and 95% ethanol. The lenses were then embedded in methacrylate JB4 (Polyscience, Warrington, PA) sectioned (2.5 μm), and stained with toluidine blue. At least three lenses from each galactose-fed group were investigated 21, 27, 30, and 33 months after the onset of galactose diet.

Results

Lenticular Aldose Reductase Activity

Aldose reductase activity in homogenates from rat, dog, and human lenses were measured by spectrophotometrically monitoring changes in the absorbance of the NADPH co-factor with DL-glyceraldehyde as substrate. In the rat lens, 5.5 nmol of NADPH were oxidized per min per mg of lens protein. Enzyme activity in the dog lens was observed to be 0.39 nmol/min/mg lens protein, a specific activity value that was approximately 14-fold lower than that of the rat lens. Similar examination of human lens aldose reductase activity also resulted in a specific activity value of 0.39 nmol/min/mg.

Progression of Cataract in Galactose-Fed Dogs

The progression of cataract formation in 30% galactose-fed dogs was periodically monitored by portable slit-lamp biomicroscopy and documented by retroillumination microscopy (Fig. 1). Within 1 month after
the onset of galactose feeding, an accentuation of anterior and posterior sutures was seen in all galactose-fed dogs before any sign of cataract. This accentuation is indicative of lens swelling and may reflect lens fiber swelling in response to the polyol accumulation. By about 3 months, vacuole formation in the equatorial region was seen (Fig. 1B). Next, in addition to cortical vacuoles, the suture regions became opaque (Fig. 1C).

Both the anterior and posterior suture regions were affected; however, posterior changes could not be clearly documented because of difficulties in photography due to the limitations of the focal plane of the camera. By 4–6 months of galactose feeding, cataract formation progressed to a third stage in which cortical opacities became visible in both anterior and posterior superficial cortical regions and gradually pro-

Fig. 1. Summary of lens changes documented by retroillumination photography of galactose-fed dogs. Photograph (A) clarity in a normal control dog whereas (B) shows the appearance of equatorial vacuoles in the cortex (A). Photograph (C) the involvement of the anterior suture region (A). Photograph (D) subcapsular anterior cortical opacities, whereas (E) shows subcapsular posterior cortical opacities. Photograph (F) clearing of the peripheral cortex (arrows) after 18 months of galactose feeding.

Fig. 2. Photographs showing lens changes in 5 (A), 12 (B), and 19-month (C) sorbinil-treated galactose-fed dogs. Note the increased appearance of clear fibers in the equatorial cortex.
gressed into the entire cortical areas (Fig. 1D). Interestingly, although these uniformly aged dogs were given equal amounts of diet, the progression of cataract showed a broad biologic variation. With slow cataract formation, the posterior involvement in the dogs was more prominent than anterior changes, and central posterior subcapsular cortical opacities were formed (Fig. 1E). These opacities were similar in appearance to those seen in human diabetes. Approximately 12 months after galactose feeding, the equatorial cortical region became demarcated and a progressive cortical clearing was seen (Fig. 1F).

The appearance of progressive cortical clearing suggests lens fiber liquefaction; yet light microscopic examination of lenses from dogs fed galactose for 21–30 months indicates that this clearing was not due to liquefaction. Examination of cataractous lenses after 21 months of galactose feeding shows that many of the lens fibers were swollen and ruptured, with large vacuoles near the bow region (Fig. 2). By 27–30 months, the width of apparently normal cortical fibers has increased (black bars). The fibrogenesis of these apparently normal lens fibers causes movement of cellular debris and aggregated lens proteins toward the border between the cortex and the nucleus.

Effect of Aldose Reductase Inhibitor Treatment on Cataract Progression

The progression of cataract formation was also evaluated in similar galactose-fed dogs concomitantly treated with the aldose reductase inhibitors sorbinil and the more potent M79175, a 2-methyl substituted analog of sorbinil. In these prevention studies, the development and progression of sugar cataract formation was arrested in an apparent dose-dependent manner. As shown in Figure 3A, none of these inhibitors, at the dosages used, prevented the formation of the initial stage of sugar cataract formation; however, clear differences in the formation of more advanced stages of cataract could be seen between the untreated and the aldose reductase-treated groups (Figs. 3B-D). The development and progression of cataract closely reflected the ability of these inhibitors to ameliorate retinal vessel changes associated with retinopathy that

Fig. 3. Percent incidence of lens changes over a 36-month period in galactose-fed dogs treated with or without aldose reductase inhibitors. Graphs (A–D) show the percent incidence of eyes from each group of dogs showing 1 or more, 2 or more, 3 or more, or 4 grade lens changes at each time interval compared with the onset of galactose feeding. Lens changes were graded as follows: 1– an incipient cataract with some degree of vacuolization in the anterior or posterior cortex; 2– a progression of vacuoles to stria in the cortex with involvement of the sutures; 3– opacification of most of the visible cortex; and 4– a complete dense cataract.

- - Untreated
- A - Sorbinil
- O - M79175 (low dose)
- M - M79175 (high dose)
in these dogs appeared in the following groups in decreasing order of frequency: no-treatment group, low-dose M79175-treated group, sorbinil-treated group, and high-dose M79175-treated group. In addition, the dose-dependent effects of aldose reductase inhibitors in preventing lens changes in galactose-fed dogs could be seen by light microscopic examination. In Figure 4, large vacuoles and swollen fibers can be seen near the lens epithelium at the bow region after 21 months of galactose feeding. At the same time, dogs receiving low doses of M79175 showed reduced vacuoles and swollen lens fibers, and the sorbinil-treated
dogs show only slight lens fiber swelling. After 27 months, large vacuoles and swollen lens fibers can still be seen; however, the width of the apparently normal superficial cortical fibers has increased. Examination of the suture areas in untreated dogs shows fiber swelling under the posterior capsule. Below these swollen fibers, dark-stained debris and ruptured fibers are visible. Dogs treated with low doses of M79175 show mild swollen lens fibers in the deep cortex and suture areas whereas those treated with high doses of M79175 appear normal near the bow area and show only slight fiber swelling near the suture. Lenses from sorbinil-treated dogs show slight lens fiber swelling at both the bow and suture areas.

After 3 months of galactose feeding, neither the low dose of M79175 nor low dose of the sorbinil appeared to prevent cataract formation. This finding led to an investigation of sorbinil plasma levels that resulted in the finding that sorbinil is rapidly metabolized in galactose-fed dogs with unanticipatedly short plasma half-life levels compared with those of either rats or humans. As a result, the amounts of inhibitors administered and the timing of each administration were increased at 4 months. In addition, a new group of 15 age- and sex-matched beagle dogs receiving 10-fold higher amounts of M79175 was added to the study. Although the increased doses of sorbinil did not reverse lens changes in these dogs, the formation of new, clear lens fibers (Fig. 5) was seen after adjustment of the doses.

**Discussion**

The osmotic (or polyol) theory of sugar cataract formation in which the aldose reductase-linked accumulation of polyols initiates the cataract formation has been established in studies of rodents. These studies indicate that the onset and progression of sugar cataract formation is directly proportional to the levels of aldose reductase seen in the lens and that their onset and progression can be prevented by the administration of suitably potent aldose reductase inhibitors.

Although the specific activity of aldose reductase in the dog lens is 14-fold lower than that in the rat lens, sugar cataracts also form rapidly in galactose-fed dogs. This cataract formation progresses from an initial accentuation of the lens sutures to the formation of equatorial cortical vacuoles, and eventually dense cortical cataracts. Light microscopy examination of these lenses shows that these cataracts are osmotic. Their onset and progression can be ameliorated in a dose-dependent manner with aldose reductase inhibi-

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**Fig. 5.** Light microscopic examination of lenses from 21–30-month galactose-fed dogs. (A) showed changes after 21 months of galactose feeding in which many of the lens fibers appear swollen and ruptured with large vacuoles near the bow region. Photographs (B) and (C) represent lenses from 27-month and 30-month galactose-fed dogs, respectively, and show the progressive widening of apparently normal cortical fibers (black bars). The fibrogenesis of these apparently normal lens fibers results in movement of cellular debris and aggregated lens proteins toward the border between cortex and nucleus (A–C × 95).
tors. Similar cataract formation was also seen with alloxan-diabetic dogs\textsuperscript{10} in that cataracts developed from an initial accentuation of the sutures to equatorial cortical vacuole formation, with eventual involvement of the suture regions to cortical opacities (data not shown). These findings suggest that sugar cataracts in the dog are also induced by the aldose reductase-initiated accumulation of polyols.

Although aldose reductase inhibitors prevent cataracts in rats, none of the doses of aldose reductase inhibitors used in this study prevented lens opacities in the dog. The dose-dependent nature of their inhibition, however, indicates that adequate amounts of inhibitors were not used, rather than the possibility that these cataracts were formed from a biochemical mechanism independent of the polyol pathway. This finding was confirmed by a similar dose-dependent observation of the progression of retinal changes and adrenal gland polyol levels.\textsuperscript{9} Although the initial stages of sugar cataracts are reversible in rodents,\textsuperscript{11} none of the early cataracts reversed with increased administration of aldose reductase inhibitors. Instead, increased administration of inhibitor caused the formation of clear, new lens fibers. The progressive formation of clear superficial lens fibers was also seen after long-term administration of galactose (>12 months). This effect appears to be independent of the adequate availability of galactose because other ocular galactose-induced changes continued to progress in these dogs.\textsuperscript{9,12} However, the possibility of decreased use of galactose in the lens must be investigated.

The appearance of cataract in the dog differs significantly from that in the rat despite the fact that both types of sugar cataracts are associated with aldose reductase. Whereas anterior cortical opacities that lead to dense nuclear cataracts predominantly occur in the rat, a more posterior cortical involvement that leads to dense cortical opacities occur in the dog. This posterior cataract is more similar to that of cataracts in human diabetic patients than to that of rat cataract. The central posterior subcapsular cortical opacity that developed slowly in the dog appears similar to cataracts often seen in diabetic patients. These results indicate that the progression of cataract does not have to be identical in different species although they are initiated by the same biochemical mechanism. Moreover, because aldose reductase activity levels in the dog lens appear to be similar to those of human lenses, our observations suggest that a similar mechanism of cataract formation may also occur in humans.

**Key words:** cataract, dog, aldose reductase, aldose reductase inhibitors, diabetes, galactosemia, and sugar cataracts

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**References**