Diabetic-Like Retinopathy in Rats Prevented with an Aldose Reductase Inhibitor

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The earliest histopathologic signs of diabetic retinopathy include selective loss of intramural pericytes and thickening of capillary basement membranes. Previous evidence from animal models indicated that aldose reductase inhibitors could prevent these capillary wall lesions, but only recently have aldose reductase inhibitors been tested for prevention of the subsequent retinal complications of diabetes, such as microaneurysms. In the present study, Sprague-Dawley rats were fed diets containing 50% galactose with or without an aldose reductase inhibitor (tolrestat). After 28 months of galactose feeding, the retinal capillaries in whole mounts exhibited a marked increase in periodic acid-Schiff (PAS) staining, extensive pericyte loss, endothelial cell proliferation, acellularity, diffuse dilation, occluded lumens, microaneurysms, and complex microvascular abnormalities including gross dilation and formation of multiple shunt networks. The PAS hyperchromaticity of basement membrane material and pericyte loss occurred throughout the retinal vasculature, while the microaneurysms and complex lesions were limited to the capillaries of the central and paracentral retina. The changes were associated with both the arterial and venous portions of the capillary plexus. Treatment with orally administered tolrestat prevented essentially all of the vessel abnormalities. Thus, long-term galactose feeding of rats induced microvascular lesions simulating those occurring in background diabetic retinopathy in humans, and these lesions were prevented by treatment with an aldose reductase inhibitor. Invest Ophthalmol Vis Sci 30:2285-2292, 1989

Diabetic retinopathy is mainly a vascular disease and primarily affects the microvasculature. As with many other ocular and systemic complications of diabetes, recent evidence indicates that the polyol pathway is involved in its etiology and that significant amelioration can be provided by treatment with aldose reductase inhibitors.1 A main limiting factor in testing the retinal effectiveness of the many potent aldose reductase inhibitors now available has been the scarcity of good animal models for diabetic retinopathy.

So far, only the alloxan-induced diabetic dog2,3 and the galactose-fed dog4,5 have been shown to exhibit retinal microvascular abnormalities closely resembling those typical of background retinopathy in human diabetes. In rats, mice, and hamsters with induced or hereditary diabetes, it has been difficult to demonstrate unequivocally diabetes-related retinal angiopathies as extensively or as consistently.6 Retinal capillary basement membrane thickening has been demonstrated in several small animal models,7 but intramural pericyte loss,8 microaneurysm formation,9-12 and other lesions have been reported only occasionally and have not been as prominent as those occurring in diabetic humans or in dog models. Even so, aldose reductase inhibitors have been reported to prevent the changes found.10

This report presents evidence that long-term galactose feeding in rats results consistently in all of the angiopaties typical of background diabetic retinopathy in humans and that this galactose-induced diabetic-like retinopathy can be prevented by an aldose reductase inhibitor. Whole mounts of the retinal vasculatures are examined here, and findings using sectioned material will be reported elsewhere.

Materials and Methods

Female CRL:COBS-CD (SD) Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington,
MA) were obtained soon after weaning and weighed 50 to 70 g. They were divided at random into three groups and were fed for 28 months a control diet, a 50% galactose diet, or a 50% galactose diet plus tolrestat (0.05% by weight; Ayerst AY-27,773, courtesy of Wyeth-Ayerst Research, Princeton, NJ). Prior to giving them the experimental diets, they were fed Purina Laboratory Chow (#5001) for 3 weeks, except for the tolrestat-treated group, which was pretreated with 0.05% tolrestat for 14 days, as described previously, to ensure complete tissue uptake of this aldose reductase inhibitor. All animals were given free access to water and food and were maintained under a 12-hr-on/12-hr-off light cycle with cage-level illuminations of 15 to 30 foot candles. Animal care and treatment conformed to the NIH Guide and the ARVO Resolution on the Use of Animals in Research.

After 28 months on the experimental diets, three rats in each group were used for the studies reported. They were killed by a lethal dose of sodium pentobarbital by intraperitoneal injection. Both eyes were removed; the right eye was fixed for light and electron microscopy (to be reported elsewhere), and the left eye was fixed for 1 to 4 days in 10% formalin buffered with 50 mM Na-K phosphate (pH 7.2) and used for vessel whole mount preparations. Following fixation, the retina of each left eye was rinsed for up to 4 hr in phosphate-buffered saline, and then all but the vascular elements were digested and removed by gentle agitation using 3.0% trypsin in 0.1 M Tris buffer (pH 7.8) at 37°C as described by Kuwabara and Cogan. Whole flat mounts of the retinal vessels thus prepared were stained by the periodic acid-Schiff (PAS) reaction; (2) dilation of veins (v); (3) increased staining but not increased diameter in arteries (a); (4) diffuse capillary dilation (cd); (5) increased capillary acellularity throughout the retina, but these appeared mainly in the central regions. Pericyte ghosts (pg) and acellular capillaries (ac) regularly occur in the vicinity of microaneurysms. Original magnifications X527 (A), X527 (B), and X330 (C) (bars = 200 µm).

Blood samples were taken upon termination of the experiment, and nonfasting blood glucose, galactose, and polyol determinations were made by gas-liquid chromatography. Diet treatments and analyses were done under masked conditions. Measurements were expressed as means ± standard deviation. Significance was determined using a student t-test.

Results

The retinal vessels of rats fed the 50% galactose diet without tolrestat for 28 months were strikingly different from those of rats treated with tolrestat, or rats fed the control diet (Fig. 1). Extensive loss of intramural capillary pericytes had occurred in the non-tolrestat group as evidenced by the presence of pericyte ghosts (basement membrane-bound outpouchings containing only remnants of degenerated pericytes) throughout the retina (Fig. 2). Hyperchromaticity (increased PAS reaction) of all vessels and a diffuse dilation of capillaries also occurred throughout the vascular system. There was an overall tortuosity of the major vessels, mainly in the venous circulation, where all vessels were enlarged as well as irregular. Measurements taken in six evenly spaced regions from central to paracentral locations along three of the major veins of retinas from each group showed an average diameter of 83 ± 5 µm in the galactose-fed rats, in contrast to 57 ± 5 µm in the control rats and 64 ± 6 µm in the rats fed tolrestat in addition to galactose (a significant increase in the untreated versus treated rats, P < 0.05). The diameters of the major arteries were indistinguishable among the diet groups, measuring 42 ± 7, 41 ± 5, and 44 ± 7 µm, respectively. There was some endothelial cell proliferation and acellularity throughout the retina, but these appeared mainly in the central regions.

The capillaries of the central retina tended to be...
Fig. 1.
more involved in angiopathy. Many of these capillaries exhibited more extensive endothelial cell proliferation, accompanied by increased dilation and aneurysm or shunt formation (Figs. 1, 3), while adjacent capillaries had become acellular and often appeared to be occluded with PAS-positive debris. Localized regions of endothelial cell proliferation and extreme dilation resulted in several microaneurysms of the fusiform (Fig. 3A) or saccular (Fig. 3B) types in the central and paracentral retina.

A very prominent pattern of angiopathy in the untreated galactose-fed rat was the occurrence of clusters of various types of complex microvascular abnormalities, mimicking those described as intraretinal microvascular abnormalities (IRMA) in both clinical and histopathologic studies of human background diabetic retinopathy16,17 and in studies of dog models.3 These formations in the galactose-fed rat exhibited marked endothelial cell proliferation, irregular diameters, and gross dilation, and involved extended lengths of capillary. Such enlarged vessels commonly formed a maze of shunts among the capillaries, extending over large portions of the central and paracentral retina (Figs. 1B, 3C). Some of these regions of hypercellularity and irregular diameters appeared to represent multiple aggregations of microaneurysms. Both the arterial and venous portions of the capillary plexus were involved. When these changes occurred near the arteries, the vessel walls became much more intensely hyperchromatic with PAS staining than they did when changes occurred near veins (Fig. 1B).

The tolrestat-treated rats exhibited essentially normal retinal vessels in spite of the 50% galactose in their diet (Fig. 1C, 2B). Capillaries of rather uniform diameter formed a plexus between each pair of arteries and veins. All vessels were slightly more PAS-positive than the vessels of control rats, presumably as a result of the PAS staining of basement membrane material, but showed much less hyperchromaticity than the untreated galactose-fed rats. Both pericyte and endothelial cell nuclei were present and clearly distinguishable in most cases. The ratio of endothelial cells to pericytes was normal for rat retinas (approximately 1.5:1.0). There were some regions with acellular capillaries in animals receiving either the control or the galactose-tolrestat diet. The galactose-tolrestat rats differed slightly from the controls in exhibiting some areas of capillary dilation accompanied by the presence of a few pericyte ghosts (two to eight per retina) and some endothelial cell proliferation. However, no more than 10% of the vessels were involved in such changes.

Tolrestat did not alter significantly the levels of blood glucose or galactose in the galactose-fed rats. At termination, the nonfasting blood glucose averaged 169 mg/dl in the rats not treated with tolrestat, 155 mg/dl in the treated, and 147 mg/dl in the controls. The galactose levels were 216 mg/dl, 235 mg/dl, and undetectable, respectively. Tolrestat did not completely normalize the blood polyol level. The average nonfasting blood polyol was 36 mg/dl in the untreated rats, 2.9 mg/dl in the treated, and less than 1.0 mg/dl in the controls. Both groups of galactose-fed rats appeared to be healthy, as judged by grooming habits, daily activity, and general appearance, but they weighed significantly less than control rats. At termination, the mean weights were 260 g, 250 g, and 566 g for the untreated, treated, and control rats, respectively.

**Discussion**

Long-term galactosemia in rats led to extensive retinal microangiopathies indistinguishable from those reported in human retinas with clinical signs of background diabetic retinopathy,16-22 and these diabetic-like retinal lesions in rats were prevented with an aldose reductase inhibitor. Success in mimicking human diabetic retinal histopathologies using a galactose-fed rat would be expected if aldose reductase were involved in diabetic retinopathy, because galactose is a better substrate than is glucose for aldose reductase, the first enzyme of the polyl pathway. Also, its product (galactitol) is not metabolized by sorbitol dehydrogenase, the second enzyme of the polyl pathway.1 Since biological membranes are probably as impermeable to galactitol as to sorbitol, much more polyol accumulates in cells of galactosemic than in diabetic rats or humans. Thus, greater metabolic disturbances and tissue toxicity related to polyl occur in the galactosemic than in the diabetic state. This results in accelerated rates of cataract formation in galactose-fed rats23 and of diabetic-like retinopathy in galactose-fed dogs3-5 compared to diabetic animals. Thus, galactosemic animal models exhibit complications resembling those characteristic of diabetes and show them sooner than do diabetic animal models.1 The acceleration of tissue damage and the general good health of the galactosemic animal makes the galactosemic model especially suitable for diabetic retinopathy and other complications of diabetes that take extraordinarily long times to develop. It would be difficult to maintain a diabetic animal for 28 months.

The retinal microangiopathies in galactose-fed rats closely resemble those typical of human diabetes in the following ways: (1) the types and spectrum of structural aberrations; (2) the occurrence in both the arterial and venous portions of the capillary plexus;
(3) the limitation of the more advanced stages to the central and paracentral retinal regions; and (4) the consistent expression of intramural pericyte loss and capillary basement membrane thickening as early histopathological changes, followed by endothelial cell proliferation, microaneurysms, and various complex microvascular abnormalities.

As in human diabetic retinopathy, intramural pericyte loss, increased PAS-positive staining, and diffuse capillary dilation appeared to be the earliest changes in galactose-induced retinopathy in rats because they were found throughout the retinal vasculature. Since proliferation of endothelial cells in some capillaries and acellularity of others were most common in the central regions, these changes probably occurred at a later stage. Alterations which involved larger segments of rat retinal capillaries, such as large microaneurysms, microaneurysm aggregates, and various complex microvascular abnormalities, including extensive shunt networks, occurred only in the central and paracentral regions. Because they were confined to the central regions and represented greater changes, these were interpreted to represent more advanced stages. As in human diabetic retinopathy, the microaneurysms and advanced microvascular abnormalities tended to occur in clusters and were surrounded in their immediate vicinity by acellular capillaries or by other capillaries exhibiting pericyte loss, hyperchromaticity, dilation, and endothelial proliferation.

The more complex microangiopathies in the galactose-fed rat retina appeared to derive from the same basic changes as did the microaneurysms, but were more developed and involved much longer vessel segments. The changes included an irregular, often extreme, dilation of the capillary wall accompanied by a hypercellularity due to proliferation of capillary endothelial cells. Although these aberrations were the same as those involved in microaneurysm formation, they were not confined to a localized area. Prominent endothelial hypercellularity and gross capillary dilation extended for great distances and involved several branches and even networks of capillaries, often resulting in multiple shunt formation. Some of the least complex changes of this type were similar to the cylindrical microaneurysm in human diabetic retinopathy illustrated by Friedenwald. 20 Such changes have been referred to as areas of ensheathing by Cogan et al. 19 Other microangiopathies appeared to represent the lesions in humans called varicosed or dilated loops. 18 Some of the aberrant formations appeared to include microaneurysm aggregates like those reported in humans. 20 Many of the more extensive alterations were probably equivalent to the tortuous, hypercellular dilated channels with proliferated endothelium which have been correlated with the clinically visible IRMA in diabetic patients. 16,17 Some IRMA leak fluorescein, and IRMA have been proposed as lesions representing attempts at neovascularization. 16

The mechanism leading to the development of diabetic-like retinal histopathologies is not understood. However, results from this and other inhibitor studies suggest that aldose reductase is involved in the process. In the present study, tolrestat prevented essentially all the various diabetic-like microangiopathies typical of background retinopathy in humans. Similar beneficial effects were produced in other models using structurally different aldose reductase inhibitors. In the galactose-fed dog model, spiro-hydantoin inhibitors were used, 9 while in fructose-fed streptozocin-diabetic rats, a carboxylic acid-type inhibitor (epalrestat) was used. 24 These findings, along with the observation that diabetic-like retinal microangiopathies could be induced by galactose feeding, strongly implicate aldose reductase in diabetic retinopathy. The similarity of galactose-induced lesions to those typical of human background diabetic retinopathy suggests aldose reductase involvement in human retinal complications.

Other evidence implicating aldose reductase in human diabetic retinopathy is the demonstration of this enzyme in human retinal pericytes in situ by immunohistochemistry 25 and in cultured pericytes by the monitoring of polyol accumulation 26 and by the detection of the messenger RNA for aldose reductase. 27 Also, primate pericytes exhibited a 3-fold increase in sorbitol and had compromised viability when incubated in a high glucose medium. 28 It has been proposed that pericyte loss could lead to loss of capillary tone, resulting in dilation, 29 and that endothelial cell proliferation may result from loss of the inhibitory control normally provided by pericytes. 30

In conclusion, the galactose-fed rat has been shown to be a good model for the histopathologies that occur with human background diabetic retinopathy, including pericyte loss, endothelial cell proliferation, microaneurysms, and various complex microvascular abnormalities. All of these lesions are prevented with an aldose reductase inhibitor. The findings from this study and from that of Kador et al 5 suggest that early treatment of diabetic patients with aldose reductase inhibitors may be helpful in delaying or possibly preventing the underlying cause of diabetic retinopathy. Now that prevention appears feasible, intervention studies on animal models are needed to provide information directly related to the design of clinical trials.
Key words: aldose reductase, diabetic-like retinopathy, capillaries, pericytes, microaneurysms, galactosemic rat model

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