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 angiopathies 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 and counter-stained with hematoxylin using the procedures described by Luna. Care was taken to maintain the same digestion and staining times and conditions for all groups of tissues processed.

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.
Fig. 3.
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 polyol pathway. Also, its product (galactitol) is not metabolized by sorbitol dehydrogenase, the second enzyme of the polyol pathway.3 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 polyol 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.3 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 com-
plex 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 be-
cause they were found throughout the retinal vascula-
ture. Since proliferation of endothelial cells in some
capillaries and acellularity of others were most com-
mon 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, in-
cluding 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 retinop-
athy, the microaneurysms and advanced microvascu-
lar 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 galac-
tose-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 dila-
tion extended for great distances and involved several
branches and even networks of capillaries, often re-
sulting in multiple shunt formation. Some of the least
complex changes of this type were similar to the cy-
lindrical microaneurysm in human diabetic retinopa-
thy 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 varicose or dilated
loops.18 Some of the aberrant formations appeared to
include microaneurysm aggregates like those re-
ported in humans.20 Many of the more extensive al-
terations were probably equivalent to the tortuous,
hypercellular dilated channels with proliferated endo-
thelium which have been correlated with the clini-
cally visible IRMA in diabetic patients.16,17 Some
IRMA leak fluorescein, and IRMA have been pro-
posed as lesions representing attempts at neovascular-
ization.16

The mechanism leading to the development of dia-
abetic-like retinal histopathologies is not understood.
However, results from this and other inhibitor studies
suggest that aldose reductase is involved in the pro-
cess. In the present study, tolrestat prevented essen-
tially all the various diabetic-like microangiopathies
typical of background retinopathy in humans. Simi-
lar beneficial effects were produced in other models
using structurally different aldose reductase inhibi-
tors. In the galactose-fed dog model, spiro-hydantoin
inhibitors were used,3 while in fructose-fed streptozo-
tocin-diabetic rats, a carboxylic acid-type inhibitor
(epalrestat) was used.24 These findings, along with the
observation that diabetic-like retinal microangiop-
athies could be induced by galactose feeding,
strongly implicate aldose reductase in diabetic reti-
opathy. The similarity of galactose-induced lesions
to those typical of human background diabetic reti-
opathy 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
immunohistochemistry25 and in cultured pericytes by
the monitoring of polyol accumulation26 and by the
detection of the messenger RNA for aldose reduc-
tase.27 Also, primate pericytes exhibited a 3-fold in-
crease 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 endo-
thelial 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, in-
cluding pericyte loss, endothelial cell proliferation,
microaneurysms, and various complex microvascu-
lar abnormalities. All of these lesions are prevented
with an aldose reductase inhibitor. The findings from
this study and from that of Kador et al5 suggest that
eyarly treatment of diabetic patients with aldose re-
ductase inhibitors may be helpful in delaying or pos-
sibly preventing the underlying cause of diabetic reti-
nopathy. Now that prevention appears feasible, in-
tervention 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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References