Absence of Degenerative Changes in Retinal and Uveal Capillary Pericytes in Diabetic Rats

Ronald G. Tilton, Lorraine S. LaRose, Charles Kilo,* and Joseph R. Williamson

Ultrastructural morphometric techniques were used to quantify pericyte degeneration in retinal and uveal capillaries of streptozotocin-diabetic rats in order to assess the suitability of this small rodent model of diabetes for studies of the pathogenesis of microvascular eye disease in diabetic humans. Male, Sprague-Dawley rats were killed by intraaortic perfusion of fixative 6 and 9 mos after induction of diabetes with 50 mg/kg streptozotocin. No differences were evident between diabetics and age-matched controls in capillary circumference, numbers of endothelial cells per capillary, and capillary cytoplasmic area of retinal, choroidal, and iridal vessels. Capillary basement membrane width and the percentage of the capillary circumference covered by pericytes were increased in retinas of diabetic vs age-matched control rats after 9 mos of diabetes (P < 0.05), but no differences were evident in the number of pericyte processes per capillary and the percentage of vessels with pericyte nuclei. No differences in pericyte distributions were observed between control and diabetic rats in the choriocapillaris and iris after 9 mos of diabetes. These findings indicate that retinal capillary basement membrane thickening precedes any evidence of pericyte degenerative changes and suggest that pericyte degeneration analogous to that associated with human diabetic microangiopathy does not occur in this experimental animal model.


Although significant advances have been made in the treatment of diabetic retinopathy, the pathogenesis of this complication of diabetes remains unclear, and it continues to be a major cause of blindness.1 A major problem in elucidating the pathogenesis of diabetic retinopathy is the lack of animal models that develop ocular lesions comparable to those in human diabetics. Diabetic monkey2-3 and dog models develop kidney and ocular lesions morphologically identical to those of humans; however, the length of time required for these lesions to develop and the cost of maintaining the animals preclude their widespread use. Although small rodents would appear to be the best compromise candidates as an experimental animal model, ocular complications typical of human diabetes are not readily produced in these models.8-9 While this may reflect a resistance to development of vascular complications, these models have not been studied sufficiently to draw definitive conclusions.

A number of retinal lesions have been reported in rats rendered diabetic with alloxan or streptozotocin, including focal and diffuse capillary basement membrane thickening,10-13 microaneurysms,10,14 pericyte loss,10,14,15 vasodilation,16 and capillary atrophy;14 however, other investigators have been unable to demonstrate such changes.17-21 Since one of the earliest morphological changes of human diabetic retinopathy is the selective loss of pericytes,22-25 the purpose of this study was to assess the extent of pericyte degeneration in retinal, choroidal and iridal capillaries of streptozotocin-diabetic rats. The latter tissues were included in this study since there is a paucity of quantitative data regarding changes in choroidal capillaries of diabetic rats13,26 and virtually no data on the iridal vasculature.

Materials and Methods

Induction of Diabetes

Diabetes was induced in male, Sprague-Dawley rats (~300 g; age ~2 mos) using 50 mg/kg streptozotocin, which was diluted in 300-μl saline, pH 5.5, and immediately injected into the left femoral vein. The animals used in this research were maintained according to the guidelines established in the ARVO Resolution on the Use of Animals in Research and were allowed pelleted food and water ad libitum. Body
weights were measured biweekly, and plasma glucose concentrations were determined monthly. Animals were killed 6 and 9 mos after induction of diabetes.

Perfusion Fixation Techniques

Rats were heparinized intraperitoneally with sodium heparin and anesthetized with sodium pentobarbital 10 min prior to death. A cannula was placed in the abdominal aorta with its tip immediately distal to the aortic arch. Ligatures were placed around the aorta and pulmonary artery at their origins; the inferior vena cava and coronary sinus were opened for venous drainage; and the animal was transferred to the perfusion chamber and connected to the arterial circuit. Following a 10-min baseline stabilization period at a perfusion pressure of 50 mm Hg, experiments were terminated by rapidly switching to fixative for an additional 15 min of perfusion at the same pressure, flow rate, temperature, pH, and P02 as the initial perfusate. The perfusate was a modified Krebs Henseleit buffer that was filtered, warmed to 37°C, and oxygenated at pH 7.4 by dialysis against 95% O2:5% CO2 immediately prior to use. The fixative was 1.25% glutaraldehyde and 1% formaldehyde in the Krebs buffer. Enucleated eyes were stored at 4°C in 0.1 M cacodylate-buffered 2.5% glutaraldehyde, pH 7.4, until processed for electron microscopy.

Tissue Processing

Eyes were cut into anterior and posterior hemispheres along the ora serrata and each hemisphere was divided into 6–8 wedge-shaped pieces, post-fixed with 1% tannic acid for 1 hr, followed by 1% osmium tetroxide (in 0.1 M cacodylate buffer, pH 7.4) for 1 hr, dehydrated with ethanol, embedded in plastic (Spurr), and polymerized at 65–75°C. Meridional sections were cut with a diamond knife using a Porter–Blum MT2-B ultramicrotome, mounted on 200 mesh copper grids, stained with uranyl acetate and lead citrate, coated with carbon, and viewed in a JEOL 100C electron microscope at 60 kv. Calibration grids were photographed daily for determination of the absolute magnification of each photograph.

Capillary Morphometry

Eight to twelve vessels were photographed randomly from each of three different tissue blocks from retina, choroid (attached to retina), and iris. In order to restrict our study to capillaries, only cross-sectioned vessels (maximum length to width ratio less than two) formed by three or fewer endothelial cells and not surrounded by vascular smooth muscle were utilized for morphometry. Retinal capillaries in the nerve fiber layer were excluded from morphometry in view of previous reports indicating that these vessels are structurally different from those of the inner and outer plexiform layers. A Hewlett–Packard computer (1200 B, Palo Alto, CA) and digitizer were used to obtain estimates of (1) capillary circumference, which was calculated by measuring the total length of the outer endothelial cell membrane, (2) endothelial cytoplasmic area, which was calculated as total area of outside circumference minus luminal and nuclear areas, and (3) the percentage of the capillary circumference covered by pericyte processes. The latter was calculated by measuring that portion of the capillary circumference in direct apposition with pericyte processes, then expressing this value as a percent of total capillary circumference. The number of endothelial cells and pericyte processes per capillary and numbers of pericyte nuclei also were enumerated. Retinal capillary basement membrane width was measured by the two-point minimum method of Williamson et al.

Statistical Analysis

All morphometric data were tabulated and analyzed with the Hewlett–Packard computer. A mean and standard deviation were determined for each parameter assessed for retina, choroid, and iris, where n represented numbers of animals evaluated. The significance of differences in capillary dimensions and pericyte distributions between diabetics and age-matched controls for each ocular tissue was assessed with Student’s t-test; the Mann–Whitney rank-sum test was used to assess differences in retinal capillary basement membrane width between controls and diabetics.

Results

Control rats doubled their initial body weights (278 ± 27 g; SD) during the first 3 mos of the study, then gained weight more slowly during the next 6 mos (Table 1). Diabetic rats gained weight at a slower rate than age-matched controls, plateauing at levels ~20% above initial, prediabetic body weights (288 ± 14 g) by 3 mos. Plasma glucose concentrations of diabetic rats were approximately 4 times age-matched control values throughout the 9 mos of the study.

Capillary Data

Numbers of endothelial cells per capillary averaged 1.6–1.7 in the retina and choroid and 2.2–2.3 in the iris of control and diabetic rats and did not vary with increasing duration of diabetes (Table 2). Capillary circumference increased in the order retina (~20 μm) < choroid (~26 μm) < iris (~33 μm); it did not vary with age and did not differ between controls and dia-

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Table 1. Rat body weights and plasma glucose concentrations

<table>
<thead>
<tr>
<th>Duration of diabetes (mos)</th>
<th>Body Weight (g)</th>
<th>Plasma glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Diabetic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>539 ± 92*</td>
<td>339 ± 60†</td>
</tr>
<tr>
<td>6</td>
<td>588 ± 110</td>
<td>340 ± 64‡</td>
</tr>
<tr>
<td>9</td>
<td>606 ± 135</td>
<td>345 ± 52‡</td>
</tr>
</tbody>
</table>

* Mean values ± SD; Student’s t-test (compared to age-matched controls): †P < 0.005; ‡P < 0.001.

betics. Likewise, endothelial area of retinal (~4 μm²), choroidal (~5 μm²) and iridial (~10 μm²) capillaries did not vary between controls and diabetics or with increasing duration of diabetes. Retinal capillary basement membrane width was ~550 Angstroms in control rats (Fig. 1) and was significantly thickened after 9 mos of diabetes (Z = 2.01; P < 0.03).

Pericyte Data

The number of pericyte processes per capillary, which was ~3 in the retina and choroid and ~12 in the iris of control rats (Table 3), did not vary with age. A small (statistically insignificant) increase in numbers of pericyte processes per capillary was observed in the retina, choroid, and iris after 9 mos of diabetes. The percentage of the capillary circumference covered by pericyte processes was ~49% in the retina, 21% in the choriocapillaris, and 57% in the iris and did not change with age in control rats. Although pericyte coverage of capillaries was significantly greater in the retina after 9 mos of diabetes (t = 2.45; P < 0.05), the magnitude of these differences was not large. In the latter tissue, pericyte coverage of capillaries was identical to age-matched controls after 9 mos of diabetes. No differences in pericyte coverage were observed between controls and diabetics in the choriocapillaris. Pericyte nuclei were associated with 14-15% of retinal capillaries, 4-8% of choroidal capillaries, and ~12% of iridial capillaries in control rats. No differences were evident between controls and diabetics in the choriocapillaris and iris; in the retina of diabetic rats, pericyte nuclei were associated with one half as many capillaries as in control retinas after 6 mos of diabetes (t = 2.75; P < 0.025), but were at control levels after 9 mos of diabetes.

Table 2. Endothelial cell data and dimensions of retinal and uveal capillaries

<table>
<thead>
<tr>
<th></th>
<th>Retina*</th>
<th>Choriocapillaris</th>
<th>Iris</th>
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<tbody>
<tr>
<td></td>
<td>6 mos</td>
<td>9 mos</td>
<td>6 mos</td>
</tr>
<tr>
<td>Control</td>
<td>126</td>
<td>122</td>
<td>116</td>
</tr>
<tr>
<td>Diabetic</td>
<td>119</td>
<td>104</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mean number of endothelial cells per capillary</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.6 ± 0.1†</td>
<td>1.6 ± 0.2</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Diabetic</td>
<td>1.7 ± 0.2</td>
<td>1.7 ± 0.2</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Capillary circumference (μm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20.1 ± 1.4</td>
<td>19.7 ± 1.1</td>
<td>27.5 ± 1.7</td>
</tr>
<tr>
<td>Diabetic</td>
<td>20.4 ± 1.1</td>
<td>20.5 ± 1.3</td>
<td>26.6 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>Endothelial cytoplasmic area (μm²)</td>
<td></td>
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<tr>
<td>Control</td>
<td>4.3 ± 0.4</td>
<td>4.5 ± 0.7</td>
<td>5.5 ± 0.7</td>
</tr>
<tr>
<td>Diabetic</td>
<td>4.3 ± 0.6</td>
<td>4.6 ± 1.1</td>
<td>5.0 ± 0.4</td>
</tr>
</tbody>
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* Vessels sampled only from inner and outer plexiform layers. † Mean ± SD; n = 6 for controls (6 and 9 mos) and diabetics (6 mos); n = 7 for diabetics (9 mos).
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diabetic rats may have several possible explanations.

Since it is unclear to what extent hereditary factors
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human diabetics include capillary basement membrane
thickening, microaneurysms, loss of pericytes, capillary
closure, and related extravascular changes such as
hemorrhages and exudates. A number of these lesions has been reported in the eyes of diabetic rats. Leuen-
berger and colleagues have described in several publications10,29,30 loss of pericytes and endothelial cells,
fusiform microaneurysms and focal capillary basement
membrane thickening after 6-12 mos in streptozoto-
cin-diabetic, Wistar rats. These changes have been
confirmed by the studies of Watanabe,31 Papachristo-
doulou et al12,14 and Studer et al.15 In contrast to the
reports cited above, other investigators have been unable
to demonstrate these capillary degenerative changes.17-21 Although we have previously demonstrated that pericyte form and distribution vary signif-
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rats,16 we were unable to detect any loss of pericytes in
these vasculatures of streptozotocin-diabetic, Sprague-
Dawley rats relative to age- and sex-matched control
rats. Thus, our observations are consistent with the
negative findings of the latter investigators cited above.
Although the possibility exists that focal areas of peri-
cyte loss could be missed with the ultrastructural tech-
niques employed in this study, we think this is unlikely
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the sex, mode of induction of hyperglycemia, and age
at the time of induction of diabetes are significant de-
terminants of the expression of diabetic microvascular
disease in this experimental animal.

We have demonstrated previously that cellular debris
within capillary basement membranes, undoubtedly
derived from pericytes, is increased significantly in
lower extremity skeletal muscles of human diabetics
compared to nondiabetics, but pericyte circumferential
coverage of capillaries does not differ between these
two groups within each skeletal muscle.33 These results
suggest that pericyte turnover is increased in skeletal
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excluded in eyes of diabetic rats since no evidence of
cellular debris within capillary basement membranes
was found in any of the capillaries used for morphom-
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Sosula et al16 have reported endothelial cell prolif-
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Although the percentage of capillaries with pericyte
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<td></td>
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</tr>
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<td>Control</td>
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Discussion

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erial diabetic rats include capillary basement membrane
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pericyte processes and pericyte coverage of capillaries were decreased in the iris of rats with diabetes of 6-mos duration, the magnitude of these differences relative to age-matched controls was not large, and the differences would appear to be of doubtful significance since corresponding data in rats with diabetes of 9-mos duration were identical to those of controls. Since the variance for the percentage of capillaries with pericyte nuclei was large, significant differences between control and diabetic rats could be detected only if large differences existed between sample means. However, the observations in the retina that pericyte coverage of capillaries was increased significantly and that the mean value for numbers of pericyte nuclei was higher in diabetic vs age-matched control rats after 9 mos of diabetes clearly suggest that pericytes were not selectively lost.

Estimates of retinal capillary basement membrane width for control rats reported in this study are virtually identical to previous reports using the same two-point minimum method of Williamson et al. The finding that retinal capillary basement membranes were thickened after 9 mos of diabetes relative to age-matched controls also is consistent with previous studies in retinas of diabetic and galactosemic rats, and attests to the effects of streptozotocin-induced diabetes on the retinal vasculature. Although some investigators have described thickened capillary walls in the choroid of diabetics, others have reported that choroidal vessels show minimal changes. We did not measure capillary basement membrane width in the choriocapillaris since strands of basement membrane material (often widely separated from one another and intermixed with collagen fibrils) are common in these capillaries, and quantitation of basement membrane area and/or width is difficult. Numerous pericyte processes surrounding individual iridial capillaries also made quantitation of basement membrane width difficult.

The demonstration of retinal capillary basement membrane thickening in galactose-fed rats suggests that capillary basement membrane thickening associated with diabetes may be linked to increased metabolism of glucose via the polyl pathway. In view of the apparent absence of pericyte degenerative changes in galactose-fed rats and in diabetic rats in this study, we would suggest that the retinal capillary basement membrane thickening in both of these conditions, as well as the recent demonstration of increased albumin permeation in ocular vessels of galactose-fed rats are most likely linked to increased polyl metabolism in vascular endothelium, resulting in endothelial cell injury and compromised vascular integrity manifested by capillary basement membrane thickening and increased vascular permeability, respectively.

In conclusion, we have demonstrated with the use of ultrastructural morphometric techniques that numbers of pericytes and their coverage of capillaries are not decreased in retinal and uveal capillaries of Sprague–Dawley rats with streptozotocin diabetes of 9-mos duration. These results suggest that (1) pericytes are not selectively lost in different ocular capillary beds of diabetic rats, and (2) this experimental animal model may not be appropriate for investigating the pathogenesis of pericyte degenerative changes associated with human diabetic microangiopathy.

Key words: pericytes, capillary, rat diabetes, streptozotocin

References