Pericyte Form and Distribution in Rat Retinal and Uveol Capillaries

Ronald G. Tilton,* Eric J. Miller,* Charles Kilo,† Joseph R. Williamson*

Ultrastructural morphometric techniques were used to assess differences in endothelial cells and in pericyte structure and distribution in rat retinal and uveal capillaries. Retinal capillaries were significantly smaller than those in the three different uveal vascular beds, all of which were similar in size. Approximately 10% of the capillaries in the retina and choroid were formed by three endothelial cells, compared with 30% and 46% of capillaries sampled from ciliary processes and iris, respectively. The percentage of the capillary circumference covered by pericytes (46–58%) and the percentage of capillary sections with pericyte nuclei (12–16%) were similar in retina, iris, and ciliary processes. Corresponding data for the choriocapillaris indicated that pericyte coverage of these capillaries was ~50% of that observed in the other eye microcirculations. The number of pericyte processes per capillary varied markedly in the different vasculatures, with an average of three for capillaries in the retina and choriocapillaris and nine to eleven for capillaries in the iris and ciliary processes. These marked differences in capillary dimensions are consistent with the well-known capillary hemodynamic and functional differences of these tissues; however, the significance of the differences in pericyte shape, frequency and distribution in the different vasculatures of the eye is less clear. Invest Ophthalmol Vis Sci 26:68-73, 1985

Because degenerative changes in retinal pericytes have been observed in association with diabetic retinopathy,1,2 these cells have been the subject of numerous light microscopic and ultrastructural studies. However, little is known regarding the normal structure and function of retinal pericytes and their role in retinal disease remains to be elucidated. Pericytes have been demonstrated in the human choroid with silver impregnation techniques3 and have been described in iridial capillaries of monkeys,4 pigs,5 and humans;6,7 however, virtually no quantitative data are available regarding their form and distribution. The paucity of information concerning pericytes is due in part to the inability to appreciate fully the structural complexity of pericyte processes with conventional light microscopic techniques and the inherent difficulty of viewing a large branching cell of this type with electron microscopy. In view of the lack of morphometric data on pericyte form and distribution in different eye vasculatures and the potential importance of such information in understanding the pathophysiology of eye diseases, we have undertaken this ultrastructural morphometric study to characterize further and compare pericytes in the retina, choriocapillaris, iris, and ciliary processes.

Materials and Methods

Perfusion Techniques

The animals used in this research were maintained according to the guidelines established in the ARVO Resolution on the Use of Animals in Research. Ten male Sprague-Dawley rats (weighing ~350 g) were heparinized intraperitoneally with sodium heparin and anesthetized with sodium pentobarbital 10 min prior to killing. A cannula was placed in the abdominal aorta with its tip immediately distal to the aortic arch. Ligatures were placed around the aorta at the cannula tip and 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 baseline stabilization period of 10 min at a perfusion pressure of 50 mmHg, experiments were terminated by rapidly switching to fixative for an additional 15 min of perfusion at the same pressure, flow rate, temperature, pH, and pO2 as the initial perfusate. The perfusate used during the stabilization period

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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% osmium tetroxide (in 0.1 M cacodylate buffer, pH 7.4) for 1 hr, followed by dehydration with ethanol, embedded in plastic (Spurr), and polymerized at 65-75°C for 48 hr. 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.

Tissue Processing

Eyes were bisected along the equatorial plane into anterior and posterior hemispheres; each hemisphere then was cut into approximately six wedge-shaped pieces, postfixed 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 for 48 hr. 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

In order to restrict our studies to capillary networks within each vasculature, 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. Capillaries with length to width ratios greater than two were considered to be tangentially or longitudinally sectioned and were excluded from analysis. By selecting vessels on the basis of the number of constituent endothelial cells and the absence of a smooth muscle layer rather than on the basis of their physical dimensions (which may be influenced by vasodilation, vasoconstriction and/or tissue processing artifacts), variation due to sampling artifacts was minimized and the likelihood increased that the vessels examined were true capillaries. Eight to twelve vessels were photographed randomly from each of three different tissue blocks from retina and choroid (attached to retina), iris and ciliary processes. A Hewlett-Packard 1000A-series computer 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 lumenal 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 vessel circumference in direct apposition to pericytes and expressing this value as a percentage of capillary circumference. Numbers of endothelial cells forming each cross-sectioned capillary, numbers of pericyte processes per capillary, and numbers of endothelial cell and pericyte nuclei also were tabulated.

Statistics

All morphometric data were tabulated and analyzed using the Hewlett-Packard computer. A mean and standard deviation were determined for each parameter assessed for retina, choroid, iris, and ciliary processes, where n represented numbers of animals evaluated. Differences in capillary dimensions and pericyte distributions between each eye tissue were assessed by using Student’s t-test and χ² analysis.

Results

Endothelial Cell Data and Capillary Dimensions

Ultrastructural features of the different capillary beds of the eye have been described by previous investigators and will not be reviewed here. The average number of endothelial cells per cross-sectioned capillary was 1.6, 1.8, 2.4, and 2.2 in the retina, choroid, iris, and ciliary processes, respectively (Table 1). Ninety three percent of all capillaries sampled

Table 1. Endothelial cell data and dimensions of retinal and uveal capillaries

<table>
<thead>
<tr>
<th></th>
<th>n†</th>
<th>Mean number of endothelial cells per capillary</th>
<th>Percentage of capillaries formed by one, two, or three cells</th>
<th>Capillary circumference (μm)</th>
<th>Endothelial cytoplasmic area (μm²)</th>
<th>Percentage of capillaries with endothelial cell nuclei</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retina</td>
<td>10 (240)</td>
<td>1.6 ± 0.2†‡</td>
<td>50 One 43 Two 7 Three</td>
<td>18.0 ± 1.2</td>
<td>3.8 ± 0.4</td>
<td>36.1 ± 10.0</td>
</tr>
<tr>
<td>Choroid</td>
<td>10 (169)</td>
<td>1.8 ± 0.1</td>
<td>32 One 58 Two 10 Three</td>
<td>27.9 ± 1.6</td>
<td>5.6 ± 0.9</td>
<td>45.2 ± 12.7</td>
</tr>
<tr>
<td>Iris</td>
<td>10 (168)</td>
<td>2.4 ± 0.2</td>
<td>3 One 51 Two 46 Three</td>
<td>28.8 ± 2.9</td>
<td>8.6 ± 1.2</td>
<td>63.4 ± 15.4</td>
</tr>
<tr>
<td>Ciliary processes</td>
<td>10 (145)</td>
<td>2.2 ± 0.2</td>
<td>11 One 59 Two 30 Three</td>
<td>28.0 ± 1.6</td>
<td>6.4 ± 0.8</td>
<td>57.8 ± 7.8</td>
</tr>
</tbody>
</table>

* Minus nuclear area.
† n represents number of rats; total number of cross-sectioned capillaries evaluated are shown in parenthesis.
‡ Mean ± SD calculated from mean values obtained from each of 10 rats.
Table 2. Relationship between number of endothelial cells per capillary and pericyte distribution in rat retinal and uveal microcirculations

<table>
<thead>
<tr>
<th>Number of cells</th>
<th>Retina</th>
<th>Choroid</th>
<th>Iris</th>
<th>Ciliary processes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of capillaries with pericyte nuclei</td>
<td>1</td>
<td>13.1 ± 8.9*</td>
<td>1.0 ± 3.2</td>
<td>25.0 ± 50.0</td>
</tr>
<tr>
<td>2</td>
<td>10.8 ± 9.2</td>
<td>6.2 ± 10.6</td>
<td>17.5 ± 15.8</td>
<td>15.3 ± 12.1</td>
</tr>
<tr>
<td>3</td>
<td>13.5 ± 19.9</td>
<td>16.7 ± 35.4</td>
<td>15.2 ± 11.9</td>
<td>22.7 ± 31.8</td>
</tr>
<tr>
<td>Mean†</td>
<td>12.1 ± 5.3</td>
<td>6.0 ± 10.0</td>
<td>16.2 ± 9.0</td>
<td>15.1 ± 8.2</td>
</tr>
<tr>
<td>Number of pericyte processes per capillary</td>
<td>1</td>
<td>3.0 ± 0.5</td>
<td>2.7 ± 0.8</td>
<td>8.3 ± 3.5</td>
</tr>
<tr>
<td>2</td>
<td>2.9 ± 0.5</td>
<td>3.1 ± 1.2</td>
<td>10.7 ± 2.3</td>
<td>8.4 ± 1.5</td>
</tr>
<tr>
<td>3</td>
<td>2.8 ± 1.0</td>
<td>5.6 ± 2.5</td>
<td>11.8 ± 2.2</td>
<td>9.0 ± 1.6</td>
</tr>
<tr>
<td>Mean</td>
<td>3.0 ± 0.4</td>
<td>3.2 ± 1.0</td>
<td>11.1 ± 2.2</td>
<td>8.6 ± 0.8</td>
</tr>
<tr>
<td>Percentage of capillary circumference covered by pericytes</td>
<td>1</td>
<td>46.9 ± 6.8</td>
<td>18.6 ± 8.2</td>
<td>58.1 ± 19.1</td>
</tr>
<tr>
<td>2</td>
<td>47.0 ± 7.5</td>
<td>22.7 ± 10.1</td>
<td>56.6 ± 8.9</td>
<td>48.9 ± 8.4</td>
</tr>
<tr>
<td>3</td>
<td>54.4 ± 16.9</td>
<td>32.6 ± 14.3</td>
<td>59.1 ± 5.9</td>
<td>43.7 ± 9.1</td>
</tr>
<tr>
<td>Mean</td>
<td>47.5 ± 6.8</td>
<td>22.0 ± 6.4</td>
<td>57.5 ± 6.7</td>
<td>46.3 ± 5.2</td>
</tr>
</tbody>
</table>

* Mean ± SD.
† Calculated from mean values of 10 rats with all capillaries formed by one, two, or three endothelial cells grouped.

from the retina were formed by one or two endothelial cells and a similar distribution, but with a slight shift toward more endothelial cells per capillary, was evident in the smallest vessels of the choroid. On the other hand, only 54% of iridial capillaries were formed by one or 2 cells, the remainder being formed by three cells. In the iris, only 3% of the capillaries were formed by one cell, compared with 32% and 50% for the choroid and retina. Capillaries of ciliary processes were similar to those in the iris, with 89% formed by two or three endothelial cells.

Retinal capillaries were the smallest, with an outside circumference of 18.0 ± 1.2 μm, and an endothelial cytoplasmic area of less than 4 μm². Uveal capillaries were significantly larger with capillary circumferences of 28 μm in the choroid and ciliary processes, and 29 μm in the iris (t = 15.65, 15.81, 10.88, respectively; P < 0.001). Endothelial area of vessels in the choriocapillaris was significantly smaller than that of iridial capillaries (t = 6.32; P < 0.001), which is consistent with the fenestrated and highly attenuated cytoplasm in the former and thick, continuous endothelium in the latter. Capillary endothelial area in ciliary processes exceeded that in the choriocapillaris (t = 2.10; P < 0.05) but was significantly smaller than that in the iris (t = 4.82; p < 0.001), reflecting the mixed "continuous/fenestrated" endothelium of these vessels. Endothelial nuclei were present in 36% of capillaries sectioned from the retina. Larger numbers of endothelial nuclei were observed in the uveal microvasculatures in the order choriocapillaris (45%) < ciliary processes (58%) < iris (63%).

Pericyte Ultrastructure and Distribution

In the retina relatively long, flat pericyte processes were separated from endothelial cells by capillary basement membrane, except at tips of pericyte processes, which usually terminated directly against endothelium. Pericytes in the choriocapillaris were similar in ultrastructural appearance to those in the retina, but were not as numerous and were separated from endothelium by strands of capillary basement membrane mixed with collagen fibrils. Tips of pericyte processes occasionally terminated as protrusions within endothelium. Large numbers of pericyte processes enclosed by capillary basement membrane were a striking feature of capillaries in the iris and ciliary processes, and many of these processes were immediately adjacent to endothelium with no intervening basement membrane. Pericyte-endothelial cell interdigitations were observed regularly.

The distribution of pericytes in retinal and uveal microcirculations is shown in Table 2. Pericytes were distributed evenly in retinal capillaries formed by one, two or three endothelial cells, with no significant differences evident in the percentage of capillaries with pericyte nuclei, numbers of pericyte processes per capillary, or the percentage of the capillary circumference covered by pericytes. An average of three processes covered 47.5% of the capillary circumference. The slight increase in pericyte coverage in retinal capillaries formed by three cells was not statistically significant. The choriocapillaris contained the fewest numbers of pericytes (only 6% of total
capillaries sectioned had demonstrable pericyte cell bodies with nuclei) and the smallest pericyte coverage of vessels (22.0 ± 6.4%). In contrast to the other capillary beds, pericyte nuclei, pericyte processes, and pericyte coverage of vessels increased in proportion to the number of endothelial cells comprising the capillary. Iridial capillaries were the most richly endowed with pericytes; 16% of all capillaries were sectioned through a pericyte cell body containing a nucleus and an average of 11 pericyte processes surrounded 46.3 ± 5.2% of the capillary circumference.

The percentage of capillary circumference covered by pericytes is shown in histogram form in Figure 1 for retinal and uveal capillaries. In the retina, approximately two-thirds of all capillaries fell within the range of 30–60% pericyte coverage, while 4–5% had less than 20% or greater than 80% coverage. In the choriocapillaris, coverage was decreased, amounting to less than 20% in one-half of the capillaries and less than 30% in three-fourths of the capillaries. Only 3% of choroidal capillaries had more than 50% of their circumference covered by pericytes. Pericyte coverage was much greater in the iris, where it exceeded 50% in two-thirds of all capillaries. This was significantly different from retinal ($\chi^2 = 26.0; P < 0.001$) and choroidal ($\chi^2 = 148.7; P < 0.001$) capillaries. No iridial capillary had a pericyte coverage of less than 10% and only 5% of all capillaries had less than 30% of their circumference covered by pericytes. Pericyte coverage in capillaries of ciliary processes was similar to that in the retina, ranging between 30 and 60% in two-thirds of the capillaries.

Numbers of pericyte processes per capillary are shown in histogram form in Figure 2 for vessels sampled from retina, choroid, iris, and ciliary processes. Pericytes were associated with every retinal capillary sampled; 69% had three or fewer pericyte processes, while no capillary had more than eight processes. Almost 14% of all capillaries sampled from the choriocapillaris were not associated with pericytes and 51% had three or fewer pericyte processes; only five of 169 capillaries had more than eight pericyte processes. Many more pericyte processes per capillary were observed in the iris than in the retina and choriocapillaris. One-third of all iridal capillaries had eight or fewer processes per capillary while two-thirds had more than eight, including 14.7% with more than 15 processes per capillary. This difference was highly significant compared with retinal ($\chi^2 = 250.5; P < 0.001$) and choroidal ($\chi^2 = 172.1; P < 0.001$) vessels. A similar distribution, but with slightly fewer pericyte processes per capillary, was observed in ciliary processes; 52% had eight or fewer and 48% had nine or more pericyte processes per capillary. This also was a highly significant difference when compared with retinal ($\chi^2 = 173.8; P < 0.001$) and choroidal ($\chi^2 = 109.7; P < 0.001$) capillaries.
Fig. 2. Histogram showing the number of pericyte processes per capillary in retina, choriocapillaris, iris, and ciliary processes. Total numbers of capillaries measured for each tissue are shown in parentheses, and the percentage of vessels within each group is shown on each bar.

Discussion

These ultrastructural morphometric data document marked differences in the morphology and distribution of pericytes in different capillary beds of the eye. Pericytes have been described encircling capillaries and venules in virtually every microcirculation composed of continuous or fenestrated endothelium, including heart, skeletal muscle, lung, connective tissue, brain, and pancreas, as well as the different eye vasculatures.\textsuperscript{1,3-5} It is not surprising that marked variations in pericyte structure, density, and distribution should be observed in different eye vasculatures, since Zimmermann\textsuperscript{8} convincingly demonstrated differences in pericyte density and form in a variety of tissues impregnated by a silver technique, later used by Wolter\textsuperscript{3} to demonstrate pericyte variations in different segments of the human choroid.

Our findings that cross-sectioned retinal capillaries are significantly smaller than uveal capillaries, and that these vessels have an extensive pericyte layer enmeshed within capillary basement membrane, are consistent with previous ultrastructural studies in rats.\textsuperscript{9-11} The presence of two or three long pericyte processes covering ~50% of the retinal vessel circumference, and the absence of demonstrable retinal pericyte processes with immunofluorescence microscopy,\textsuperscript{12} suggest that these processes are not “tubular,” but rather, have a flat “pancake” appearance. The demonstration of pericytes in the choriocapillaris is consistent with the reports of several investigators,\textsuperscript{13-16} although their paucity may account for several reports that they are absent in this tissue.\textsuperscript{17-19} The observation that pericytes are virtually absent from choroidal vessels formed by one endothelial cell is of interest since one-third of all capillaries in this microvasculature consists of only one cell.

Although the ultrastructure of iridial capillaries has been described previously,\textsuperscript{4,5,7} few investigators have noted the abundance of pericytes and extensive pericyte coverage of these capillaries, which equal or exceed those in the other capillary beds examined. Saari\textsuperscript{5} described only “membranous contacts” between pericytes and endothelium in the pig iris, while Vegge\textsuperscript{4} described endothelial cell-pericyte interactions in the Vervet monkey similar to those that we have reported. Since the number of pericytes (reflected in the percentage of capillaries with pericyte nuclei) is similar in the iris and ciliary processes, the smaller number of pericyte processes per capillary and the smaller pericyte coverage of vessels in the latter tissue indicate that these cells are less branched. It is of interest that pericyte density, form, and distribution are similar in capillaries of the iris and ciliary processes (relative to other ocular microcirculations), yet endothelial cell structure differs significantly.

Three categories of endothelial cell-pericyte interactions have been described: (1) pericyte processes in close apposition to endothelium with no intervening basement membrane, (2) pericyte processes that protrude into endothelium, and (3) endothelial processes that protrude into pericytes. Examples of the first category are observed regularly in retinal and uveal capillaries. In the retina, tips of most pericyte processes terminate directly against endothelium, and in the iris and ciliary processes, many of the numerous pericyte processes also are found immediately adjacent to endothelium. Penetration of endothelium by pericyte processes is observed in all eye vasculatures but
is much more frequent in the uveal microcirculations. This type of interaction has been described previously in the retina and choroid\textsuperscript{20} and in skeletal muscle.\textsuperscript{21} The penetration of endothelial processes into pericytes observed in the uveal vasculature also has been reported in the choroid and retina,\textsuperscript{20} as well as in skeletal muscle\textsuperscript{21} and cerebral cortex.\textsuperscript{22} The significance of these pericyte-endothelial cell interactions remains unclear but may represent points of communication between these two cells and/or anchoring sites.

In conclusion, our morphometric data indicate that retinal capillaries are significantly smaller than uveal capillaries (all of which are of comparable size), and that endothelial cell morphology and pericyte shape, frequency and distribution differ markedly in the retina, choriocapillaris, iris, and ciliary processes. The large number of highly branched pericytes in the anterior uveal microcirculations and their intimate structural relationship to endothelium suggest that pericytes may play an important functional role in these microcirculations.

Key words: pericyte, endothelium, retina, uvea, ultrastructure

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References