Endothelial fenestrae in proliferative diabetic retinopathy

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The ultrastructure of old neovascular preretinal membranes was examined in both eyes of a patient with proliferative diabetic retinopathy treated in one eye with photocoagulation. Membranes in both eyes consisted of a matrix rich in mature collagen surrounding viable new vessels and "ghost vessels." Viable vessels of different calibers frequently showed endothelial fenestrae bridged by diaphragms. Occasionally tight junctions between endothelial cells appeared altered. Fenestrae and incompetent junctions may account for the characteristic "leakiness" of newly formed vessels.

Key words: proliferative diabetic retinopathy, blood-retinal barrier, fenestrae, tight junctions, fluorescein angiography

A characteristic of newly formed blood vessels in proliferative retinopathies is their excessive leakiness to fluorescein.1 So great is the permeability of these vessels that a green haze may be seen with special filters several minutes after intravenous injection of fluorescein.

We wondered whether it was possible to demonstrate in proliferated retinal blood vessels a morphologic basis for this permeability by studying in an electron microscope preretinal neovascular tissue from a diabetic patient. Interestingly, we found frequent fenestrations of endothelial cells and occasionally apparent alterations of their junctions.

Materials and methods

A 59-year-old man had proliferative diabetic retinopathy 11 years after the diagnosis of diabetes, with new vessels superiorly and on the disc in the right eye and new vessels inferonasally and on the disc in the left eye. Both eyes had repeated episodes of vitreous hemorrhage. The right eye later developed elevated new vessels and preretinal fibrovascular membranes. It was left untreated, whereas the left eye received focal xenon arc photocoagulation to patches of new vessels. The treatment was followed by partial regression. Fluorescein angiography identified several arteriolar feeder vessels of the disc's new vessels. Five sessions of focal argon laser treatment were carried out, but the vessels recurred and extended nasally, forming a large fibrovascular membrane from the disc toward both nasal quadrants. Peripheral xenon scatter photocoagulation was then applied and was followed by another partial regression of new vessels.

Nineteen months later the patient died from a cerebrovascular accident. We obtained his eyes at autopsy, opened them through the pars plana, fixed them in 4% buffered glutaraldehyde, pH 7.4, and dissected from them preretinal fibrovascular tissue. We took tissue samples from the superior periphery of the right (untreated) eye and from the nasal part of the large membrane of the left (treated) eye (Fig. 1). The tissue samples were...
Fig. 1. Fundus photograph of left eye showing fibrovascular membrane (fvm) overlying disc (D) and extending nasally and inferiorly. Tissue was sampled from the farthest nasal (=left) extension of the membrane and is shown microscopically in Fig. 2, top.

embedded in Epon-Araldite, and thick sections were cut at 1.5 \( \mu m \) for light microscopic orientation. Thin sections were obtained and examined in an electron microscope.

Results

Light microscopic cross-sections of the preretinal fibrovascular membranes showed numerous vascular channels of varying diameters embedded in a fibrous matrix (Fig. 2). Some channels were formed by viable endothelial cells lining a lumen that contained red blood cells. Other channels were acellular with fibrous material filling part or all of their lumen ("ghost vessels").

Ultrastructurally the viable channels were formed by endothelial cells and pericytes (Figs. 3 to 5). Multiple layers of basement membrane were associated with the outer circumference of both endothelial cells and pericyte processes, but between endothelial cells and pericyte processes basement membrane material was usually reduced to one or two sheets. The outer basement membrane layers blended into the fibrous interstitial matrix that contained numerous mature collagen fibrils identifiable by their characteristic periodicity of 640 A (Fig. 3).

In a number of blood vessels of the preretinal membranes of both eyes the endothelial cytoplasm focally tapered off to form fenestrae bridged by a diaphragm (Figs. 3 to 5). The diaphragm was slightly thinner than the adjacent plasma membrane and contained a central density (Fig. 4, bottom; Fig. 6, top). Although endothelial pores were usually closed by one one-layered diaphragm, occasional fenestrae with two one-layered diaphragms were also seen (Fig. 6, bottom). Upon measuring the width of 20 diaphragms, we obtained a mean value of 605 A, ±108 (S.D.), range 400 to 800 A. Fenestrae with diaphragms were seen along endothelial areas that bordered the fibrous matrix around the vessel as well as areas which bordered pericyte processes. Endothelial discontinuities without a closing diaphragm were not encountered.

The endothelial cells of most blood vessels were connected by tight junctions showing periodic fusions of the outer leaflets of their plasma membranes. However, in some in-
Fig. 2. Top, Membrane of left (treated) eye consists of vascular channels embedded in a fibrous matrix (fm). bv, Viable blood vessels; gv, ghost vessels. A smooth cellular surface (sm) lines vitreous aspect of the membrane. Bottom, Fibrovascular membrane of the fellow right (untreated) eye also contains viable blood vessels and ghost vessels. R, Retina. (Light micrographs; toluine blue.)

stances the outer leaflets were merely approximated without periodic fusions (Fig. 7).

Comment

The neurosensory part of the blood-retinal barrier consists of vascular endothelial cells with a thick continuous cytoplasm and of tight junctions between adjacent endothelial cells. During fluorescein angiography the normal barrier retains the dye, allowing the retinal blood vessels to remain distinctly visible. In proliferative diabetic retinopathy, neovascular formations profusely leak fluorescein, obscuring the clear demarcation of the blood vessels. In our study preretinal neovascular formations showed a morphologic change of the blood-retinal barrier; that is, the endothelial cytoplasm was not thick and continuous but thin and interrupted by fenestrae bridged by diaphragms. In addition, occasional junctions between adjacent endothelial cells were lacking the typical morphologic “tight” appearance.

Fenestrated new blood vessels were found among the new vessels of both the treated and untreated eyes. Thus the formation of fenestrations was not dependent upon the effects of photocoagulation treatment.

Fenestrated capillaries occur normally both elsewhere in the body and in the eye. Elsewhere in the body they are found mainly underneath the absorptive or secretory epithelia of the gastrointestinal tract and within kidney and endocrine glands. Fenestrae have
Fig. 3. Electron micrograph. Viable blood vessels of membrane in right (untreated) eye consist of endothelial cells (end) joined by tight junctions (tj) and pericytes (p). Endothelium forms fenestrae near short arrows at left. Note multiple layers of basement membrane (bm) and microfilaments of pericytes near long arrows. The stroma is rich in mature collagen fibers (col).

an average pore diameter of approximately 600 Å in cross-sectioned material and 685 Å in freeze-etch preparations, with a range of 520 to 1000 Å. Most fenestrae are permanently or transiently spanned by a diaphragm consisting of two centrally located fibrous rings connected by radiating fibers to the outer leaflet of the plasma membrane of the pore rim. The regional frequency of fenestrae varies among different organs. Electron-dense tracers of 50 to 300 Å particle size readily penetrate fenestrae within minutes after intravenous injection, indicating that fenestrae most likely serve a special function in allowing rapid transfer of fluid and small molecules. In the eye only the capillaries of the choroid and ciliary body are fenestrated, mainly on the side facing Bruch’s membrane. The tracers ferritin, silver nitrate, and horseradish peroxidase reportedly escape from choriocapillaries, mainly through fenestrae, to impregnate Bruch’s membrane.

Fenestrated capillaries arising in pathologic conditions have rarely been studied. Normally nonfenestrated muscle capillaries
Fig. 4. Top, At higher magnification endothelial fenestrae (f) and tight junctions (tj) show typical configuration. Bottom, Upon suitable sectioning a central density (arrows) is visible within the diaphragms spanning endothelial fenestrae.

Fig. 5. Fenestrae (arrows) in blood vessel of membrane of left (photocoagulated) eye.
Fig. 6. At higher magnification most fenestrae show typical configuration (top), but occasional double diaphragms are also seen (bottom).

Fig. 7. Endothelial junction of blood vessels in membrane of left eye lacks periodic fusions of outer leaflets of plasma membrane. A singular fusion may be suspected near arrow. L, Lumen of blood vessel; bm, basement membrane.
form fenestrations during wound healing and become three to four times more permeable than normal vessels to substances of 70,000 molecular weight.8 In the eye, new vessels in retina and iris were said to have a fenestrated endothelium.9 Fenestrations were shown in rubeosis iridis resulting from diabetes10 and from sickle cell disease11 and in intraretinal vessels resulting from urethane retinopathy.12 We know of only one instance in which fenestrae have been documented previously in preretinal blood vessels of proliferative diabetic retinopathy.13

Physiologically, fenestrae correspond to sites of increased permeability. The function of fenestrations in pathologic conditions is unknown. It has been suggested that they may be "weak spots" responsible, for example, for anterior chamber hemorrhages in rubeosis iridis.11 Interestingly, neither regenerating fenestrated capillaries in muscle nor normal fenestrated capillaries in the choroid are known to bleed spontaneously. Both have in common a mechanical support by the surrounding tissue. By contrast, fenestrated new iris vessels and particularly fenestrated preretinal new vessels in proliferative diabetic retinopathy may lack support by surrounding tissue.

Incompetent endothelial junctions may be another factor weakening the wall of preretinal new blood vessels in proliferative diabetic retinopathy. In our case, after extensive searching, junctions were found which seemed to lack the periodic fusions of the outer leaflets of adjacent plasma membranes characteristic for tight junctions of normal retinal blood vessels. Discontinuous junctional elements were also seen on freeze-fracture studies of fenestrated blood vessels of the normal ciliary body14 in cross-sections of proliferated iris vessels11 and upon testing junctional tightness with the electron microscopic tracer horseradish peroxidase in the following experimental conditions: in intraretinal blood vessels during experimental hypertension13 and during chronic nonproliferative diabetic retinopathy,16 in cerebral vessels upon osmotic stress,17 and in immature capillaries of the spinal cord proliferating after mechanical injury.18

It is difficult to assess the respective roles of diabetes and vascular immaturity in the formation of widened tight junctions of blood vessels studied in this report. Many of the blood vessels were embedded in a thick collagenous scar. They had the large luminal diameters of venules, were surrounded by numerous basement membrane rings, and had many distinct pericytes. These features are characteristically seen in mature rather than immature vessels and suggest that the diabetic condition per se was responsible.

Fenestrations and widened tight junctions may be expected to alter the exchange mechanism between preretinal new vessels and the surrounding vitreous, creating a new "blood-vitreous barrier." It is tempting to speculate whether or not this might facilitate or fail to inhibit growth of additional new vessels.

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REFERENCES
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