ORIGINAL ARTICLES – Laboratory science

Ultrastructure of retinal vessels in diabetic patients

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Abstract

The ultrastructure of retinal vessels was examined in three eyes from diabetic patients and two eyes from control subjects. In some of the retinal blood vessels of each of the diabetic eyes, the endothelial cytoplasm was thin and had fenestrae. These fenestrations which were seen in both capillaries and venules, were closed by a thin diaphragm. Transendothelial channels were also observed in the endothelial cytoplasm. The tight junctions between endothelial cells were rarely altered. The junctions were open with short adherent regions in which the junctional membranes showed increased electron density. These morphological changes may account for the characteristic breakdown of the blood-retinal barrier in diabetic patients.

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Retinal vessels of human and animal eyes are characterised by non-fenestrated continuous endothelial cells with tight intercellular junctions, which are reported to form the blood-retinal barrier (BRB).^{1,2} Breakdown of the BRB is one of the hallmarks of diabetic retinopathy, and is responsible for macular oedema, the most common cause of visual morbidity in diabetic patients.³ BRB breakdown has been demonstrated by fluorescein angiography and vitreous fluorophotometry in diabetic humans^{4,5} and rats,^{7,8} and results of ultrastructural studies suggest that breakdown of the tight junctions^{9,10} or increases in vesicular transport¹⁰ might contribute to the increased permeability in the retina. Viores *et al*^{11,12} reported that immunohistochemical staining for albumin was useful in localising BRB breakdown in human diabetic retinas because, as albumin is one of the serum proteins, its extravascular localisation signifies breakdown of the BRB. Recently, we showed immunohistochemical detection of extravasated fibrinogen (fibrin) in human diabetic retina.¹³ However, the cellular mechanisms that lead to the increased permeability of the retinal vessels of diabetic patients are unknown. In the study described herein, the morphological changes of diabetic retinal vessels, with special reference to their permeability characteristics, were studied by transmission electron microscopy.

Materials and methods

Three eyes were surgically obtained from three diabetic patients (patients 1, 2, and 3) because of intractable ocular pain caused by neovascular

glaucoma. Two control eyes were also surgically obtained but from patients (patients 4 and 5) without diabetes mellitus. One of the control eyes was enucleated because of neovascular glaucoma after central retinal artery occlusion, and the other was enucleated because of chronic angle closure glaucoma.

Immediately after enucleation, a 5 mm penetrating incision was made at the pars plana, and the eyes were placed in 4% glutaraldehyde with 0.1 mol/l phosphate buffer ($\text{KH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4=1/3$, pH 7.2) for 24 hours. Each eye was then bisected at the equator and small pieces of retinal tissue with choroid and sclera in the posterior pole were trimmed using a razor blade. Following several rinses with the same buffer solution, the tissues were postfixed in 1% OsO_4 buffer with 0.1 mol/l phosphate. After dehydration in a series of graded alcohols, the tissues were embedded in Epon 812. Semi-thin sections (1 μm thickness) were cut on an ultramicrotome (Porter-Blum MT II, Sorvall, Newtown, USA), were stained with azur II and examined with a light microscope. Thin sections were cut on the same ultramicrotome, stained with uranyl acetate and lead citrate, and examined with an electron microscope (JEM-100 CX, Jeol, Tokyo, Japan). Some lesions were also sectioned serially for electron microscopy.

Case reports**PATIENT 1**

This 59-year-old man had been diagnosed with non-insulin-dependent diabetes mellitus (NIDDM) at age 46. The diabetes was poorly controlled, even with insulin. In 1974, at age 53, he visited our clinic with complaints of visual disturbance. Visual acuity in the right eye was no light perception and in the left eye, 10/200. Ocular tension of the right eye was 64 mm Hg and of the left eye, 15 mm Hg. Slit-lamp examination revealed rubeosis iridis of the right eye, which was diagnosed as neovascular glaucoma. Ophthalmoscopic examination showed proliferative diabetic retinopathy of both eyes. Panretinal photocoagulation was performed in the left eye, and panretinal photocoagulation and cyclocryotherapy were performed in the right eye. Ocular tension remained high, however. Because of persisting ocular pain, nausea, and headache, the left eye was enucleated in 1980.

PATIENT 2

This 31-year-old man had been diagnosed as

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Figure 1 (A) Low power electron micrograph ($\times 2000$) of inner retina of patient 1. A capillary (C) that has thickened basal lamina is present in the inner retina. An occluded vessel (O) is seen near the capillary (C). Glial cell proliferations are observed in the retinal parenchyma. (B) Higher magnification ($\times 44\,500$) of the capillary shown in (A). The endothelial cytoplasm is thin and has a fenestra (arrowhead) that is bridged by a thin diaphragm. (C) Serial section ($\times 43\,000$) of the capillary shown in (B). The diaphragm of the fenestra contains a central density (arrowhead). (D) Higher magnification ($\times 44\,600$) of the capillary shown in (A). The endothelial cytoplasm has a fenestra that is closed by double diaphragms (arrowhead). ILM inner limiting membrane. L, lumen of capillary.

having insulin-dependent diabetes mellitus (IDDM) in 1964, when he was 20 years of age. However, he refused medical intervention. He visited our clinic in 1974 because of visual disturbance and ocular pain. Visual acuity in the right eye was no light perception, and in the left eye, 14/20. Ocular tension of the right eye was 69 mm Hg, and of the left eye, 17 mm Hg.

Slit-lamp examination showed rubeosis iridis of the right eye, which was diagnosed as neovascular glaucoma. Ophthalmoscopic examination revealed proliferative diabetic retinopathy of both eyes. Panretinal photocoagulation was performed on the left eye; photocoagulation was not performed on the right eye because of corneal opacity and posterior synechiae. Because the ocular pain persisted, the right eye was enucleated in 1975.

PATIENT 3

In 1963 at age 40 this man was diagnosed as having NIDDM. In 1981 he visited our clinic (age 59 years). Visual acuity in the right eye was 20/20, and in the left eye, 12/20; ocular tension

Figure 2 (A) Low power electron micrograph ($\times 1300$) of inner retina of patient 2. A capillary (C) has thickened basal lamina and the lumen contains erythrocytes. (B) Higher magnification ($\times 36\,500$) of the capillary shown in (A). The endothelial cytoplasm has a fenestra that is closed by a thin diaphragm (arrowhead). The endothelial cytoplasm also contains cytoplasmic vesicles, some of which fuse the plasma membrane of the endothelial cell (arrows). L, lumen of capillary; P, pericyte.

Figure 3 (A) Low power electron micrograph ($\times 1900$) of inner retina of patient 1. A venule (V) is formed by endothelial cells (E), pericytes (P), and thickened basal lamina (B). The lumen contains erythrocytes. Glial cell proliferations are present around the venule. (B) Higher magnification ($\times 43\,400$) of the venule shown in (A). The endothelial cytoplasm has two fenestrae that are closed by thin diaphragms (arrowheads). (C) Higher magnification ($\times 43\,500$) of the venule shown in (A). The endothelial cytoplasm has a fenestra that is bridged by double diaphragms (arrowhead). ILM, inner limiting membrane; L, lumen of venule.

Figure 4 (A) Electron micrograph ($\times 11\,300$) of retinal capillary of patient 2. The capillary wall consists of endothelial cells (E), degenerative pericytes (P), and thickened basal lamina (B). The endothelial cells are connected by intercellular junctions. (B) Higher magnification ($\times 35\,400$) of the capillary shown in (A). The intercellular junction is open, with short adherent regions in which the junctional membranes show increased electron density (arrow). L, lumen of capillary.

Figure 5 (A) Electron micrograph ($\times 6000$) of retinal venule of patient 3. The wall of the venule is formed by endothelial cells (E), pericytes (P), and thickened, laminated basal lamina (B). The pericyte (P) has a electron lucent cytoplasm. (B) Higher magnification ($\times 43\,600$) of the venule shown in (A). The endothelial cells are connected by open intercellular junctions with short adherent regions in which the plasma membrane has a layer of finely filamentous material on the inner surface (arrow). L, lumen of venule.

of the right eye was 18 mm Hg and of the left eye, 15 mm Hg. Ophthalmoscopic examination and fluorescein angiography showed preproliferative diabetic retinopathy in the left eye, and pan-retinal photocoagulation was performed. About 1 month later, visual acuity in the left eye was perception of hand movement, and ocular tension was 43 mm Hg. Slit-lamp examination showed rubeosis iridis.

Ophthalmoscopic examination revealed central retinal artery occlusion as well as diabetic retinopathy. Despite drug therapy and cyclo-cryotherapy, ocular tension of the left eye remained high and visual acuity was no light perception. Trabeculectomy and peripheral iridectomy were carried out in an attempt to alleviate the ocular pain due to neovascular glaucoma. Unfortunately, the ocular pain persisted, and the left eye was enucleated in 1982.

PATIENT 4

In 1980, a 78-year-old woman was diagnosed as having central retinal artery occlusion and neovascular glaucoma. In 1988, when she was 86 years old, she first visited our clinic with complaints of left ocular pain and headache. Visual acuity in the left eye was no light perception and ocular tension was 43 mm Hg. Slit-lamp examination of the left eye revealed a corneal ulcer, rubeosis iridis, and cataract. The left fundus was invisible by ophthalmoscopy. Because ocular tension of the left eye remained elevated, and ocular pain persisted, the eye was enucleated in 1988.

PATIENT 5

In 1974, at the age of 73, this woman had undergone intracapsular lens extraction for a

senile cataract of the right eye. Following lens extraction, ocular tension of the right eye increased, and remained elevated despite intensive drug therapy. In 1986, at age 85, she first visited our clinic because of pain in the right eye. Visual acuity in the right eye was no light perception and ocular tension was 54 mm Hg. Slit-lamp examination revealed corneal opacity and ulcer, shallow anterior chamber, and posterior synechiae. The ocular fundus could not be visualised by ophthalmoscopy. Following a diagnosis of chronic angle closure glaucoma and because of persisting ocular pain, her right eye was enucleated in 1986.

Results

Retinal blood vessels located in the inner retina at the posterior pole were examined by electron microscopy (Figs 1–5). The retinal vessels of diabetic eyes (patients 1, 2, and 3) were formed by endothelial cells, pericytes, and thickened basal lamina (Figs 1A, 2A, 3A, 4A, 5A). The pericytes occasionally showed degenerative changes that were characterised by electron lucent areas within the cytoplasm (Figs 4A, 5A). In a few retinal vessels of each of the diabetic eyes, the endothelial cytoplasm focally tapered off to form fenestrae (Figs 1B–D, 2B, 3B, C). Both capillaries and venules were fenestrated. The diameters of the fenestrae ranged from 50–60 nm, and each was closed by a thin diaphragm. The diaphragm was slightly thinner

than the adjacent plasma membrane, and some contained a central density (Fig 1C). Although fenestrae were usually closed by a single one-layered diaphragm, occasional fenestrae with two one-layered diaphragms were also seen (Figs 1D, 3C). Fenestrated endothelial cells contained intracytoplasmic vesicles (Fig 2B). The retinal parenchyma in the region of fenestrated vessels was usually atrophic, containing glial cell processes (Figs 1A, 3A).

The endothelial cells of most vessels from the diabetic eyes were connected by tight junctions showing fusion points between their plasma membranes. However, a small number of capillaries and venules had open interendothelial junctions with one or two short adherent regions in which the plasma membrane had a layer of finely filamentous material on the inner surface (Figs 4B, 5B).

The retinal vessels of the control eyes (patients 4 and 5) had no fenestrated endothelial cells. These endothelial cells were connected by tight intercellular junctions.

Discussion

In the present study, at least some retinal vessels from each of the diabetic patients showed morphological changes that could affect the BRB. Specifically, the endothelial cytoplasm was not thick and continuous, as is seen in normal eye vessels but, rather, was thin and interrupted by fenestrae that were bridged by diaphragms. It is widely accepted that vascular endothelial cells are labile and can change their structural characteristics in response to various circumstances and to some agents, and that endothelial fenestrations are observed in the attenuated portions of the endothelial cells, where the thickness is equal to or less than the diameter of cytoplasmic vesicles.^{14,15} Regarding formation of the fenestrae, Elfvin¹⁶ suggested that the cytoplasmic vesicles might pass through several transitory stages in their development. It may be that one stratum of the double layered membranes of a vesicle, one layer of which faces the lumen and one of which faces the basal lamina, might eventually disappear, leaving only single layered membrane in the vascular wall. The development of fenestrae might presumably occur also after fusion of apposing areas of the endothelial plasma membrane. In the present study, it was also demonstrated that fenestrae with two one-layered diaphragms were also aligned across endothelial cytoplasm, thereby suggesting the formation of transendothelial channels.¹⁷

Since endothelial fenestration and transendothelial channels are an anatomical property related to high permeability for water and electrolytes, and also for passage of large molecules,^{17,18} our study suggests that the fenestrated portions and transendothelial channels of the retinal vessels might represent areas with increased permeability. To our knowledge, fenestrated vessels arising in pathological conditions have rarely been studied. McKinney *et al.*¹⁹ reported that normally non-fenestrated muscle capillaries did form fenestrations during wound healing, and became three to four times more permeable than normal vessels. In the eye, new

vessels located in the preretinal proliferative tissue of diabetic patients were shown to have fenestrated endothelial cells.^{20,21} However, because the fenestrated vessels in the present study were located in the retina, and had thickened basal lamina and degenerative pericytes, identical to the typical morphological appearance of retinal vessels in diabetic patients,^{22,23} it is suggested that these vessels are not regenerating or newly formed vascular channels. Fenestrated vessels in the retina have been described in Coat's disease,²⁴ phototoxic retinopathy,²⁵ and radiation retinopathy.²⁶ However, transendothelial channels were not observed in retinal vessels.

The function of the fenestrations that are seen in pathological conditions is unknown. Bellhorn²⁷ hypothesised that a controlling factor in the development of fenestrated vessels is the metabolic demand of tissues. This, however, seems an unlikely explanation for the fenestrations in retinal vessels of diabetic patients, since there is no apparent significant metabolic needs of gliotic retinas.

Another morphological change affecting the BRB was the incompetent endothelial junctions of retinal vessels. In our study, some junctions were open, with short adherence regions where junctional membranes showed increased electron density. These junctions, which are classified as intermediate junctions, are known to present no effective barrier to the penetration of the intercellular cleft.²⁸ These altered intercellular junctions of retinal vessels have been seen when junction tightness was tested under experimental conditions of diabetes^{9,10} and hypertension²⁹ with electron microscopic tracers such as horseradish peroxidase and microperoxidase.

The three diabetic eyes that had morphological changes that would cause increased permeability showed not only diabetic retinopathy, but also had glaucoma, and it is suggested that glaucoma may accelerate or perpetuate such morphological changes. However, since control eyes that also had glaucoma did not show the morphological changes of increased permeability, it is apparent that the formation of fenestrations and the altered intercellular junctions are not dependent upon the effects of glaucoma.

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