Retinal vessel abnormalities of phototoxic retinopathy in rats

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Phototoxic retinopathy in rats is characterized by a progressive loss of the outer retinal layers. During the neural degenerative phases, a sequence of vascular change occurs. Whole mounts of ink-injected retinal vessels and flat mounts of trypsin-digested retinal vessels show areas of capillary nonperfusion and acellularity and enlargement of retinal capillaries in the deep bed. When the photoreceptor and outer nuclear layers have disappeared, portions of the remaining deep capillary bed become surrounded by cells of the retinal pigment epithelium (RPE). Thin sections show fenestrae in some vessel walls within the retinal pigment epithelial layer. Since serial sections show continuity between those fenestrated vessels and the deep retinal vessels and no evidence of vessels breaching Bruch’s membrane, this morphologic phenomenon is considered to be an in situ alteration of retinal vessels. We conclude that a factor(s) within the retinal pigment epithelial layer determines the morphology of vessels within that environment.

Key words: retinal vessels, blood-retinal barrier, phototoxic retinopathy, rat, permeability, retina, electron microscopy, fenestrations, capillaries

The retinal vessels of laboratory rats, like those of man, are characterized by continuous endothelial cells with “tight” intercellular junctions which are reported to form the inner blood-retinal barrier. In contradistinction, the choriocapillaris is characterized by fenestrated endothelial cells, and the outer blood retinal barrier component is found at the tight junctions of the retinal pigment epithelium (RPE).

Studies of urethan-induced retinopathy in rats demonstrated a degeneration of the outer retinal layers, angiographic evidence of sodium fluorescein leakage, and fenestrated vessels within the RPE. Because we had not observed any blood vessels crossing Bruch’s membrane, we postulated a modification of the typical retinal capillary.

Blood vessels within the RPE have been observed in hereditary and phototoxic rat retinopathies and also in an age-related retinopathy in Fischer rats. The investigators did not indicate that the vessel in the RPE layer connected to the underlying choriocapillaris, with one exception.

Angiographic studies in our laboratory of late-stage urethan-induced and phototoxic rat retinopathies demonstrated leakage of sodium fluorescein and decreased leakage of fluorescein-labeled dextrans when larger molecules were utilized. Because both retinopathies are characterized by vessels within the RPE, we hypothesized that fenestrated vessels would be present within the RPE in the phototoxic dystrophy as well. In order to begin testing this hypothesis and to establish
Fig. 1. Photomicrograph of rat retina showing decreased width of outer nuclear layer and decreased length of photoreceptors. (Periodic acid-Schiff stain; ×800.)

the origin of those vessels, we undertook the following investigations.

Materials and methods

Experiment 1. Eighteen female Long-Evans rats (100 to 150 gm) were placed in clear plastic cages and exposed for 96 hr to 300 ft-cd of fluorescent light (measured at cage floor). Ocular pigmentation in this strain of rat markedly protects the eye from brightness, so pupillary dilation was maintained with 1 drop of 1% atropine sulfate twice a day. The rats were provided laboratory feed and water ad libitum. Following exposure, the rats were returned to a cyclic light environment. Two rats were sacrificed at 2-week intervals from weeks 8 to 24 after exposure. Prior to sacrifice, the eyes were examined by indirect ophthalmoscopy, fundus photography, and routine sodium fluorescein angiography. At sacrifice, eyes were selectively prepared for (1) light microscopy using Zenker's fixation and paraffin embedding, (2) flat mounts of trypsin-digested retinal vessel, or (3) whole mounts of ink-injected retinal vessel.

Experiment 2. Three female Long-Evans rats that had been exposed to light 10 to 12 months previously were sacrificed, and eyes were prepared for electron microscopy according to our standard laboratory procedure. After hemisection through the optic nerve head, the posterior ocular segment was serially sectioned at 3 μm, and some vessels within the RPE were chosen for electron microscopic evaluation. The selected 3 μm thick sections were re-embedded for thin sectioning by placing an inverted gelatin capsule containing epoxy resin over the tissue and allowing it to polymerize at 60°C. If fenestrated endothelium was found, the adjacent serial sections were examined to ascertain the origin of the vessel. The course of the vessel was charted in three dimensions by overlaying tracings of vessel profiles from projected photomicrographs.

Results

Experiment 1. Histopathologic variation occurred between paired rats and even between eyes from a single rat, presumably the result of light exposure differences due to behavioral characteristics. The relationship
between the vascular and neural abnormalities, however, was quite consistent, and the data are based upon that interrelationship.

The neural retinopathy progressed in a pattern consistent with that previously described, i.e., a progressive and sequential loss of the photoreceptors and the outer nuclear and outer plexiform layers (Fig. 1). In general, this sequence of events began in the peripapillary region and spread peripherally.

After loss of the outer neural retinal layers, the inner nuclear layer came into apposition with the RPE. Where the RPE was absent, the inner nuclear layer made contact with Bruch's membrane, and cystic spaces in the inner nuclear layer and inner plexiform layer were frequently evident in these areas. However, an occasional cystic space was observed in areas with RPE.

The following vascular changes were associated with the retinopathy:
1. As the photoreceptor and outer nuclear layers began to diminish in thickness, whole mounts of ink-injected tissue revealed a coil-like appearance of precapillary arterioles in the peripapillary area of the retina and an enlargement of some deep capillary bed drainage venules. No marked vascular alterations or leaks of sodium fluorescein were seen by fluorescein angiography. Some pigment mottling of the fundus was observed ophthalmoscopically, but histologic alterations of the RPE were not evident at this stage.

2. As the deep retinal capillary bed came in proximity to the RPE, more extensive vascular abnormalities became apparent. Fluorescein angiography and whole mounts of ink-injected retinal vessels demonstrated areas of capillary nonperfusion and associated shunt vessels (Fig. 2). No leakage of sodium fluorescein was observed angiographically. Trypsin digest preparations showed areas where acellular capillary strands were prevalent. Endothelial nuclei were clustered in

Fig. 2. Whole mount of ink-injected retinal vessel showing area of nonperfusion (A) in deep capillary bed and adjacent enlarged drainage venule (arrow). (x125.)
Fig. 3. Photomicrograph of rat retina showing marked loss of photoreceptors and outer nuclear layer, and markedly enlarged vessel in outer plexiform/inner nuclear layer. Note normal sized capillary above the enlarged vessel. (Hematoxylin-eosin stain; x800.)

precapillary arteriolar branchings, possibly as a result of migration from the acellular capillary strands. Enlarged capillary and/or venular profiles were evident in the outer plexiform layer (Fig. 3).

3. When the outer nuclear layer was absent, the deep capillary bed was brought into contact with the remaining pigment epithelial cells and some vessels became enveloped by the RPE (Fig. 4). By migration and/or proliferation, the cells of the RPE extended along vessels connecting the inner retinal arteries and veins to those vessels within the RPE. As long as a single layer of photorecep-
Fig. 4. Photomicrograph of rat retina showing complete loss of outer nuclear layer and photoreceptors. Vessel profiles (V) extending from inner nuclear layer into retinal pigment epithelial layer are evident. (Hematoxylin-eosin stain; ×800.)

Tyne cell nuclei intervened, no interaction between the deep capillary bed and the RPE was evident. Throughout this microscopic study, no evidence of a vessel crossing Bruch's membrane was observed.

Ophthalmoscopically the retinal vessels appeared narrowed, but fluorescein angiography showed the blood column caliber to be normal, although marked areas of nonperfusion and tortuous shunt vessels were observed. No leakage was observed except in one rat (20 weeks after exposure) in which slight peripapillary leakage was detected.

Experiment 2. Light microscopic examination of plastic sections from the posterior ocular segment showed a marked late-stage phototoxic retinopathy characterized by a loss of the outer retinal layers, disorganization of the inner nuclear layer, large vessel profiles extending from the inner retina to clusters of
vessel profiles within the RPE, and proliferation and/or migration of RPE cells along those vessels transversing the retina (Fig. 5). In some areas there was total dropout of RPE cells with inner retina glial tissue adjacent to Bruch's membrane. In other areas, the RPE cells were hypopigmented to varying degrees. It was not unusual to find vessels surrounded by these nonpigmented cells.

The electron microscopic appearance of the vessel profiles within the RPE was varied, and individual vessels did not consistently exhibit all the characteristics to be described. In general, they were of small caliber and found as clusters within a small area (Fig. 6). When vessels were enveloped by one or more cells of the RPE, the basal side of the cell abutted on the vessel. The RPE cells themselves, in general, did not degenerate, and junctional contacts between cell apices were usually present. No alterations of the choriocapillaris were observed.

Some of the vessel profiles within the RPE were typically choroidal in that the endothelium was thin whether it was fenestrated or not (Fig. 6). The basement membrane was less wide than that of a retinal capillary and tended to be separated from the membrane of the endothelial cells by a clear space of variable dimensions. There were junctional contacts between the endothelial cells, and it was not unusual to find fenestrations (Fig. 6). Sometimes there was a series of fenestrations (Fig. 7), and other times there was a single fenestrated area where the membrane of the endothelial cell was clearly condensed.

Other vessels within the RPE appeared typically retinal, with thick endothelial cells joined by tight junctions and surrounded by a
Fig. 6. Electron micrograph of three vessel profiles clustered within the pigment epithelium. An intact Bruch's membrane is seen at B. The large patent vessel with two red blood cells is fenestrated (FV), and the other patent vessel has a continuous endothelium (CE). The third vessel has a swollen endothelial cell (E) and may be degenerating. The pigment epithelial cells are irregularly arranged around the vessels, indicating either a movement or new cell synthesis. Pigmentation is light in this area. (×4500.)

thick basement membrane closely apposed to the cell membrane of the endothelial cell and pericyte (Fig. 8). They contained the small mitochondria and rough endoplasmic reticulum and somewhat elongated nucleus typical for a retinal capillary. Both the retinal and choroidal-like vessels had patent lumens, and frequently red blood cells were found within the lumen, indicating that these were active vessels.

Between those two vessel profile types, i.e., the retinal and choroidal type, there were other vessel profiles which were not clearly one or the other. For example, the basement membrane of some vessel profiles appeared to be laminations of several layers.
of thin basement membrane. Some vessels showed degenerated pericytes within their basement membrane; others exhibited large amounts of rough endoplasmic reticulum and polysomes in combination with an irregular nucleus. This latter observation, true for vessels with and without fenestrae, may be an indication of new endothelial cell synthesis. Finally, there were also vessels that appeared to be typical retinal capillaries in almost every respect, except that one or two fenestrae were present (Fig. 8).

In three eyes, the course of a fenestrated vessel within the RPE was traced by evaluation of the serial sections. In each instance, they connected only to vessels originating from the inner retinal layers. There was no evidence by either light or electron microscopy of any crossing of Bruch’s membrane by any vessel within the RPE.
Fig. 8. Capillary between two pigment epithelial cells (1 and 2) which is surrounded by basal cell processes. The continuous endothelial cells are thick and joined by junctional complexes. The basement membrane is relatively thick. Despite these characteristics, three fenestrae are clearly visible (arrow). (x9500.)

Within the RPE, accumulations of basement membranelike material were also observed. These accumulations appeared to represent the end product of a degenerating vessel.

Discussion

The present investigation of rat phototoxic retinopathy demonstrates that the deep retinal capillary bed is brought into close proximity to the RPE as a consequence of degeneration of the outer neuroretinal layers. As the deep capillary bed makes contact with the RPE, there is a movement of cells of the RPE away from their normal position, and the new cells are unable to synthesize pigment, which accounts for the observed non-pigmented RPE cells. The shifting of RPE cells results in a surrounding of the capillary in a manner whereby the basal side of the
RPE cell is in contact with the vessel basement membrane. Several sequelae are possible. First, the retinal capillary may totally degenerate; second, the retinal capillary may retain its normal structure and patency; third, the capillary may lose pericytes and acquire additional laminations of basement membrane material from the enveloping RPE cell; and fourth, the capillary endothelial cells are stimulated into formation of new vessels. It is possible that in either the third or fourth situation, the vessels may develop fenestrations. We have established that these fenestrated vessels are retinal vessels that have undergone a morphologic alteration rather than growing in from the choriocapillaris. This phenomenon is now known to occur in two rat retinal degenerations (urethane-induced and phototoxic). Since several hereditary rat retinopathies have a similar end-stage pathology, it is probable that this phenomenon also occurs in those diseases.

The sequence of neural and vascular changes in experiment 1 was similar to that previously reported for albino rat phototoxic retinopathy except that there was no mention of vessels within the retinal pigment epithelial layer. In a similar study on hereditary rat retinopathy in pigmented animals, that phenomenon was noted. In the phototoxic study with albino rats there may have been complete destruction of RPE, as compared to our study using pigmented rats. Certainly, our study showed RPE pathology only in later phases of the retinopathy.

An increased O2 tension in the degenerating outer retinal layer has been hypothesized as the cause of the vaso-oblitervative changes. Oxygen, however, is known to have an obliterative effect on immature retinal vessels but only a constrictive effect on mature retinal vessels. It is possible that vaso-obliteration occurs because of the degenerating outer neural layers; however, we have previously demonstrated a lack of morphologic abnormalities in retinal vessels within an inner retinal layer degeneration. Thus it may be a combination of high O2 tension and degenerating neural cells that causes vaso-obliteration to occur.

Gerstein and Dantzker suggested that as capillary dropout and presumed nonperfusion occur, adjacent vessels become shunt vessels as a means of expediting the arteriovenous flow. Then, as the deep capillary bed comes closer to the choriocapillaris, capillary degeneration increases until the O2 tension within the remaining tissues is tolerable rather than excessive. We might expect therefore a total obliteration of the deep capillary bed as the outer retina completely degenerates. However, we did not observe a total obliteration of the deep capillaries but rather an enveloping of some of those capillaries by cells of the RPE and an alteration of their morphology. We believe that the vessel morphology is determined by the requirements of the tissue it subserves. Thus, if a vessel is placed in an unusual environment, the morphology of that vessel may change. For example, a renal carcinoma which metastasized to the brain became vascularized by fenestrated blood vessels arising from the normally nonfenestrated cerebral vessels.

Conversely, Grindle and Marshall recently reported the presence of a nonfenestrated vessel in the subretinal (not subpigment epithelial) space in a case of senile macular degeneration, a vessel that had arisen from the choroid. Thus this vessel, within the environment of the neural retina, assumed a characteristic of retinal vessels.

What is the mechanism responsible for the altered vascular morphology in this rat retinopathy? One possibility is that these fenestrated vessels within the RPE may represent immature vessels as part of a neovascularization process. However, fenestrations are a characteristic of a mature choriocapillary type vessel, not an immature vessel. The immature choriocapillaris is nonfenestrated, and fenestrations occur at or about the same time the RPE cells develop tight intercellular junctions. It is possible that the immature retinal capillary has a thick continuous endothelial lining and a patchy basement membrane. The abnormal vessels in this rat retinopathy have a thin, fenestrated endothelium and a complete basement membrane. Thus they appear morphologically similar to mature choriocap-
illaris and not an intermediate stage in retinal vessel development.

The clinicopathologic correlation portion of this present study did not continue past the stage where vessels within the RPE became a prominent feature. We think that the fenestrated vessels within the retinal pigment epithelial layer account for at least part of the leakage of sodium fluorescein observed by us in previous studies.\(^6\)\(^{17}\) It is also possible that leakage may occur from the choriocapillaris into the retina through areas where the RPE layer is absent. We previously described\(^17\) two types of leakage in outer layer rat retinopathies. The first type was a patchy fluorescence that did not progress in size. This could represent contained leakage from fenestrated vessels within the RPE. Even though the RPE cells had undergone proliferation and/or migration, the RPE barrier to fluorescein could remain intact during mitosis.\(^29\) Also, the patchy fluorescence became less evident as higher-molecular-weight fluorescein-labeled (FITC) dextrans were utilized,\(^17\) and it is possible that the larger-sized molecules did not pass through the fenestrations.

The second type of leakage was a diffuse peripapillary fluorescence that increased in magnitude with time when sodium fluorescein or low-molecular-weight FITC dextrans were used but was less obvious with higher-molecular-weight dextrans. Such fluorescence could come from leakage from the choriocapillaris where the retinal pigment epithelial layer was absent and be decreased due to a discrimination against the larger dextran molecules by the choriocapillaris fenestrations. This question is currently under further investigation using fluorescence microscopy.

The importance of this vascular morphologic phenomenon in understanding basic vessel response is evident, but it also has marked importance in clinical ocular disease. Taniguchi\(^30\) has reported the presence of fenestrated vessels in proliferative tissue in late-stage diabetic retinopathy, and Taniguchi et al.\(^31\) have reported the presence of fenestrated small retinal vessels in diabetic retinopathy. Fenestrated radial peripapillary capillaries have also been observed by Ueno and Matsuo\(^29\) in human eyes with diseases including glaucoma and diabetic retinopathy. Thus fenestrated retinal vessels are directly associated with clinical ocular disease, and it is obviously important to the treatment of clinical disease that we understand the mechanism of these vessel permeability and morphologic alterations. The outer retinal layer degenerations in rats may provide a meaningful model for studying this phenomenon.

REFERENCES

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