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Microvessel loss, vascular damage and glutamate redistribution in the retinas of dogs with primary glaucoma

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Abstract

Objective Vascular damage and ischemia-like changes in glutamate distribution occur in primary glaucoma (PG) in dogs. We measured the microvessel density in PG retinas to determine whether microvessel loss may induce ischemia and glutamate redistribution.
Animals studied Sections from 12 control and 33 glaucomatous dog retinas.
Procedures Vessels in retinas were identified by staining with *Griffonia simplicifolia* isolectin B4 or immunohistochemical staining for laminin or glutamate. Damage to regions of the inner nuclear layer (INL) was classified as mild (< 10% damaged neurons), moderate (≥ 10% damaged neurons, INL ≥ 2 cells thick) or severe (INL < 2 cells thick).
Results Glutamate redistribution was found in some mildly damaged regions and increased as damage increased. Regions with increased glutamate redistribution and increased damage had lower densities of microvessels in plastic sections. However, neuronal damage and glutamate redistribution were seen even in areas adjacent to the remaining microvessels. Microvessel loss in damaged regions was confirmed in paraffin sections with lectin staining and immunohistochemical localization of laminin. The density of larger vessels was not decreased in PG, but larger vessels often had thickened walls, cuffing with leukocytes, and leakage of albumin.
Conclusions Microvessel loss may occur in regions of glutamate redistribution and neuronal damage in PG retinas. Larger vessels were often damaged. The redistribution of glutamate is associated with a loss of microvessels, even in mildly damaged regions. However, neuronal damage and glutamate redistribution may occur close to remaining microvessels, suggesting microvessel loss was not the sole factor inducing these changes.

Key Words: blood vessels, dogs, glaucoma, glutamate, microvessels, retina

INTRODUCTION

Glaucoma is characterized by a progressive loss of retinal ganglion cells that is often associated with increased intraocular pressure[1,2] and decreased retinal blood flow.[3–5] Progressive damage to other types of retinal cells also occurs in primary glaucoma (PG) in dogs, including cells of the inner nuclear layer (INL) and outer nuclear layer (ONL).[6,7] Cell loss with focal thinning of these layers eventually occurs in PG in dogs.[6,7]

Several studies suggest there may be altered blood flow and damage to blood vessels in the retinas of dogs with glaucoma. Damage to retinal blood vessels occurs in dogs with PG.[8] These damaged vessels have thickened walls containing deposits that are labeled by periodic acid Schiff stain. Beagles with open-angle glaucoma also have structural abnormalities in the endothelium of capillaries of the lamina cribrosa.[9] Gelatt *et al.* have reported that altered ocular blood flow occurs before intraocular pressure is elevated in Beagles with open-angle glaucoma.[10]

The altered blood flow and vascular damage reported in canine glaucoma might lead to ischemia and contribute to retinal damage in canine PG. PG-induced damage is often focal, and may involve all neuroretinal layers.[6,7] Neuronal loss is greatest in the inner retina, which is consistent with ischemic damage.[7] A redistribution of glutamate[7,11] and taurine,[11] also consistent with ischemia, is greater in the more severely damaged regions. The redistribution consists of a loss of glutamate from neurons and an accumulation of glutamate in glial cells. We hypothesized that this focal

pattern of changes may be due to ischemia, which is induced by microvessel losses such as those that occur in retinopathy of prematurity,[12] diabetic retinopathy,[13] and some retinal degenerative diseases.[14,15]

In the current study we tested the hypothesis that vascular damage may lead to microvessel obliteration and focal ischemia. We compared the microvessel densities of retinal regions with differing degrees of damage and glutamate redistribution to determine if microvessel loss may contribute to glutamate redistribution and retinal damage in PG.

METHODS AND MATERIALS

Acquisition and processing of eyes

Retinal samples were of two types: (1) plastic-embedded retinas which were acquired for immunohistochemical studies of glutamate distribution, and (2) archived, paraffin-embedded retinas (acquired by RRD), which were used for identification of blood vessels using lectin staining and immunohistochemical localization of laminin. The retinas that were plastic embedded were from dogs with PG undergoing enucleation as part of treatment. Control plastic-embedded retinas were obtained from dogs euthanized at a local humane Society. A complete ophthalmic examination (slit-lamp biomicroscopy, binocular indirect ophthalmoscopy, and applanation tonometry) was performed prior to euthanasia and found to be normal in each control dog. Eyes were obtained with adherence to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and with approval from the Animal Care and Use Committee of Colorado State University.

The 10 plastic-embedded glaucomatous retinas were acquired from several veterinary ophthalmologists and from the Colorado State University Veterinary Medical Center. In the plastic-embedded eyes, no more than two globes were obtained from any single breed. The 23 paraffin-embedded glaucomatous samples were obtained from 19 Cocker Spaniels, three Basset Hounds, and one Dachshund. The duration between presentation of the dogs to ophthalmologists for clinical signs and subsequent enucleation was known for the archived specimens. Retinas acquired ≤ 5 days after onset of clinical signs were considered acute, while retinas acquired > 5 days after onset of signs were considered chronic.

Fixation and sectioning

Plastic-embedded specimens were fixed as previously described.[7] Eyes were rapidly dissected and the dorsotemporal quadrant of the globe was then placed in 0.3% glutaraldehyde with 4% paraformaldehyde in 0.05 M phosphate buffered saline (PBS). Quarters were then processed and embedded in Epon as previously described.[11] Paraffin-embedded retinas were fixed by immersion in either 4% buffered formalin or Bouin's fixative/ethanol, without penetration of the globe. The samples were embedded in paraffin and sagittally sectioned at a 5-μm thickness.

Staining of paraffin sections

Microvessel counts. Paraffin sections from PG and control dog eyes were stained by two methods to determine microvessel numbers per unit length of retina. Sections from each eye were immunohistochemically stained for laminin using an avidin-biotin method. In brief, sections were dewaxed in xylene and graded ethanol. Sections underwent antigen retrieval by autoclaving for 15 min in a 0.1 M citrate buffer, pH 6.0. After this endogenous peroxidase was inactivated in 3% peroxide for 15 min, sections were incubated in primary rabbit antisera to laminin (1 : 100 in PBS; Dako Cytomation, Carpinteria, CA, USA) overnight. A Vectastain elite kit for rabbit antisera (Vector Laboratories, Burlingame, CA, USA) was then used to visualize the primary antibody using peroxidase with diaminobenzidine as chromogen. Other sections were stained with biotinylated *Griffonia simplicifolia* isolectin B4 (Vector Laboratories). Sections were processed as described for laminin immunohistochemistry, with biotinylated lectin diluted 1 : 100 substituting for the primary antibody. The biotinylated lectin was visualized with the ABC component of the Vectastain kit, with metal-enhanced diaminobenzidine (Sigma-Aldrich, St Louis, MO, USA) as chromogen. All slides were dehydrated through graded ethanol, cleared in xylene, and coverslipped.

Albumin and CD3 immunohistochemistry. Sections from each eye were processed essentially as described above for laminin immunohistochemistry using rabbit primary antibodies to albumin or rabbit primary antibodies to CD3 (Dako), and diluted 1 : 400 in PBS for albumin or 1 : 50 in PBS for CD3.

Staining of plastic-embedded eyes

Postembedment immunogold and toluidine blue staining were performed on adjacent, 0.5-μm sections using the same methods we have previously employed.[11,16] Serial semi-thin sections (0.5 μm) of retinas embedded in Epon were made using an ultramicrotome. Some sections were stained for 20 min at 60 °C with 1% toluidine blue in 1% sodium borate buffer. Other, near-adjacent sections were etched and immunogold stained for glutamate using the method for silver-intensified immunogold labeling suggested by Chemicon International (Temecula, CA, USA) for their antisera to amino acids. Sections mounted on gel-coated slides were etched with sodium ethoxide diluted 1 : 5 in ethanol for 20 min, rinsed in ethanol and water, and blocked with 5% normal goat serum diluted in 0.05 M PBS. Sections were rinsed in PBS and incubated overnight in 1 : 400 dilutions of rabbit antisera to glutamate (Sigma-Aldrich) in PBS. After rinsing in PBS, sections were incubated for 4 h in secondary gold-labeled (ultrasmall particles) anti-rabbit or anti-mouse antisera (Electron Microscopy Sciences, Hatfield, PA, USA) diluted in PBS (1 : 20). Sections were rinsed for 30 min in PBS and then in two rapid changes of water. Staining was silver enhanced for 6 min, and the slides were then rinsed in water and coverslipped for image capture. Digital images were

captured using a Zeiss Axioplan 2 microscope with Axiovision 3.1 software (Carl Zeiss MicroImaging, Inc., Thornwood, NY, USA). For measurements of staining density, images were analyzed using ImageJ 1.34 (National Institutes of Health, Bethesda, MD, USA). Controls for specificity included omission of the primary antibody and inhibition of staining by pre-incubation of antisera with glutamate conjugated to bovine serum albumin (absorption controls).

Assessment of damage

Regions of INL in glaucomatous retinas were classified as mildly damaged, moderately damaged or severely damaged by the estimated percentage of damaged cells and the thickness of the INL in the region in both paraffin and plastic sections. Plastic sections were stained with toluidine blue and paraffin sections were stained with hematoxylin and eosin to evaluate damage. Mildly damaged regions contained ≤ 10% damaged INL cells and an INL thickness of ≥ 2 cells. Moderately damaged regions contained > 10% damaged cells and an INL thickness of ≥ 2 cells. Severely damaged regions showed thinning of the INL to < 2 cells in thickness. Damaged INL cells were defined as those with a dark, condensed nucleus and/or swollen, pale cytoplasm. Regions with highly variable thickness of INL or variable percentages of damaged cells were not included in the study unless at least 70% of the region fitted one of the categories.

Statistical analysis

Quantitative data on microvessel density and quantitative immunohistochemical staining densities obtained from plastic sections were analyzed for statistical significance using ANOVA followed by Tukey's multiple comparison tests using GraphPad Instat 3 Software (San Diego, CA, USA).

RESULTS

Glutamate redistribution in PG

In plastic sections the densities of glutamate immunostaining of cells and INL regions were measured in a semiquantitative manner using an immunogold technique. Control retinas showed high levels of glutamate immunoreactivity in putative neurons of the INL, with much lower levels in putative Müller cells (Fig. 1a,e).

Regions with different degrees of damage were evaluated for glutamate redistribution in plastic sections. Damage to INL cells in PG may progress from mild damage, where a normal number of cells are still present but some have clumping of chromatin and pale, swollen cytoplasm, to more severe damage where the loss of cells results in thinning of the INL.[7] To determine the stage of damage at which microvessels are lost, damage to INL regions was classified as mild, moderate or severe. Cells were classified as damaged if they had dark nuclei with condensed chromatin or pale, swollen cytoplasm.

The densities of glutamate immunostaining in putative neurons of the INL were decreased in damaged regions (Fig. 2). The average level of glutamate immunoreactivity was measured in 50-μm-long areas of INL in regions with different degrees of damage. The average density of INL glutamate immunostaining decreased as the severity of damage increased (Fig. 2a). In contrast to neurons, the average level of glutamate immunostaining was increased in putative Müller cell bodies of the INL and in Müller cell processes of the nerve fiber layer (Fig. 2b) in a similar manner to the glutamate redistribution we have previously described.[7,11] Within mildly damaged regions of the INL, 50-μm areas were seen that had either near-normal levels of glutamate (Fig. 2a; Mild, High Glu) or significantly decreased levels of glutamate (Fig. 2a; Mild, Low Glu). When control retinas were examined for similar focal regions of glutamate loss from the INL, none were seen except in regions with obvious sectioning or staining artifacts. Quantitative measures of staining density for glutamate in control retinas confirmed that regions with the lowest levels of glutamate staining on visual inspection were not significantly different from adjacent regions with higher levels of glutamate staining ($P > 0.4$; data not shown).

Microvessel densities were decreased in regions with glutamate redistribution and neuronal damage

Microvessel densities in plastic sections were measured using glutamate immunoreactivity as a marker for endothelial cells. As shown in Fig. 3, endothelial cells of the vessels maintained substantial levels of glutamate at all severities of damage, allowing glutamate immunostaining to be used as a marker to identify microvessels.

The densities of microvessels in PG retinas were significantly decreased in regions with increased severity of damage (Fig. 4a) and lowered neuronal glutamate staining densities (Fig. 4b). Microvessels were identified by the high levels of glutamate immunoreactivity maintained in endothelial cells, even in severely damaged regions (Figs. 1,3).

Microvessel obliteration was confirmed using other markers for blood vessels

To confirm that a microvessel loss occurred in PG, other methods of labeling vessels were used. Staining with *Griffonia simplicifolia* isolectin B4[17] or antibodies to laminin[18,19] have often been used to identify blood vessels.

Sagittal sections from paraffin-embedded retinas of dogs with PG and control dogs were stained with lectin or immunohistochemically stained for laminin to measure microvessel densities. We were unable to use either lectin staining or immunohistochemical staining for laminin successfully on plastic sections. Leukocytes and macrophages are also labeled with the lectin.[20] To ensure that leukocytes outside of blood vessels were not counted as microvessels in the lectin-stained retina, labeled structures needed to have an identifiable lumen to be considered a microvessel. No significant differences were found between the numbers of microvessels in sections stained with lectin or antibodies to laminin in controls ($P > 0.6$; data not shown). Typical staining

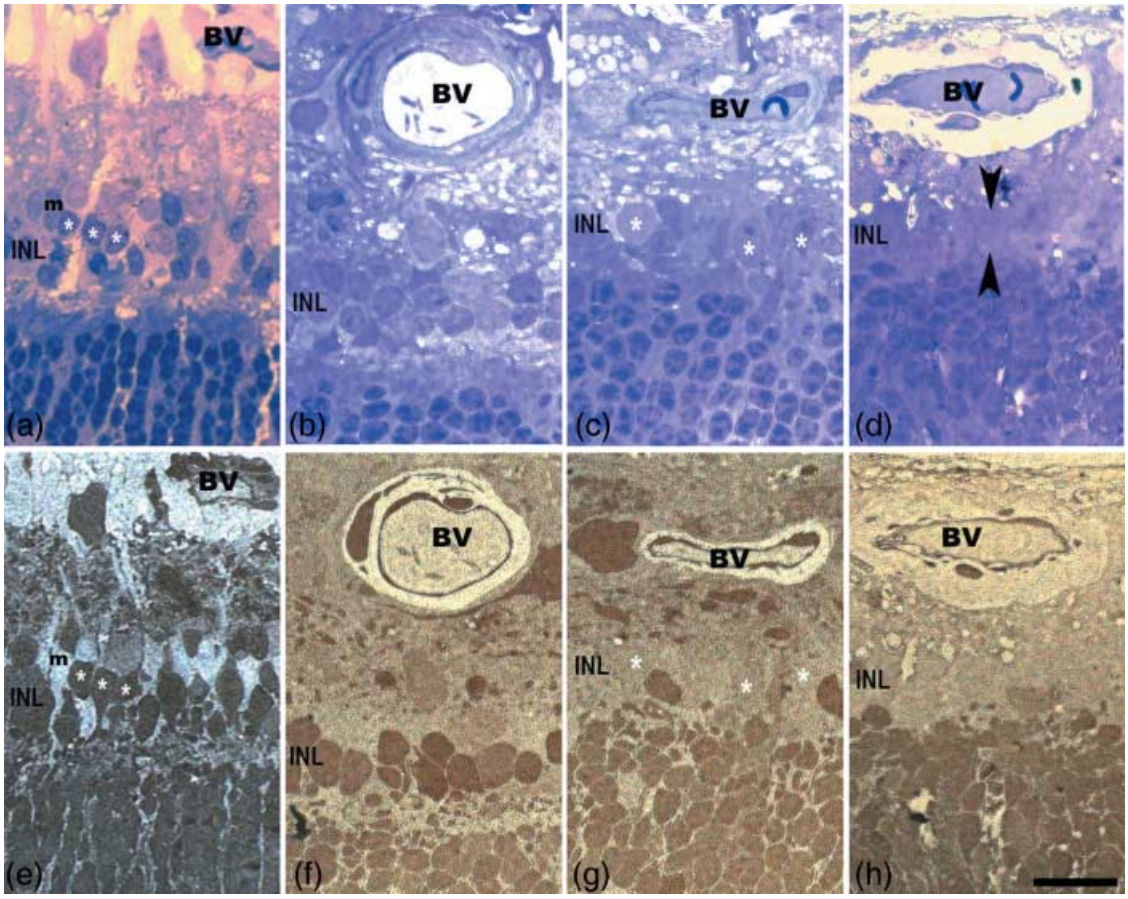

Figure 1. Glutamate redistribution occurred in regions with different severities of PG damage. (a–d) Morphologic appearances of the INL in regions with different severities of damage are shown in toluidine blue-stained sections. (e–h) Glutamate immunostaining of adjacent 0.5-µm sections from the same regions shown in a–d. (a) Control section. Typical undamaged neurons of the INL are indicated by asterisks. m, typical Müller cell; BV, microvessel. (b) A region with mild damage and high glutamate levels. (c) A region with mild damage and low glutamate levels. Almost all neurons appear undamaged (asterisks). (d) A region with severe damage. Note thinning of the INL (between arrowheads). (e) Glutamate immunostaining of the same control region of the retina as (a). Note the high levels of glutamate immunoreactivity in the INL neurons (asterisks), also labeled in (a) and a low level of glutamate in the Müller cell (m), also labeled in (a). (f) Glutamate immunostaining of the same region as (b) with mild damage and high levels of glutamate. Note the large number of neurons with high levels of glutamate immunoreactivity. (g) Glutamate immunostaining of an adjacent section of the same region as (c) with mild damage and low levels of glutamate. Note the low levels of glutamate in some undamaged neurons (asterisks). (h) Glutamate immunostaining of the same region as (d) with severe damage. Note the low levels of glutamate immunoreactivity in the thinned INL. All images are shown with the same orientation and magnification. Bar = 15 µm.

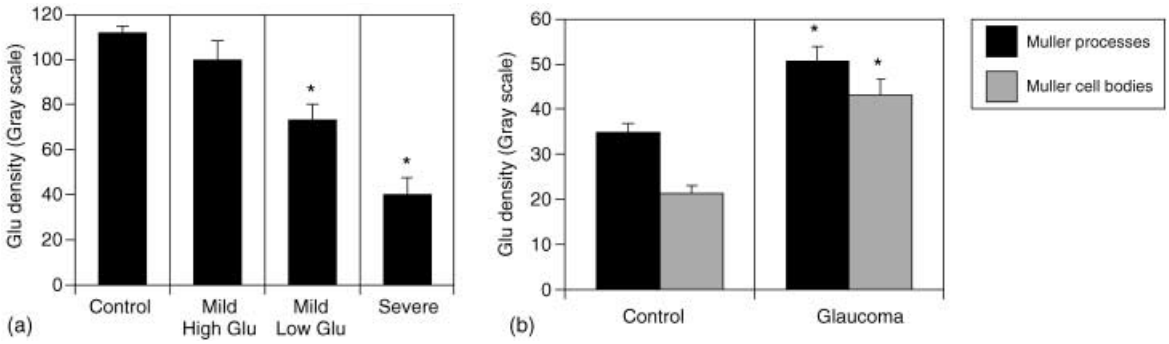

Figure 2. Glutamate redistribution was dependent on the severity of damage in PG. (a) The average glutamate immunostaining density in 50-µm regions of the INL was reduced as the severity of damage increased. Within mildly damaged regions, smaller areas ≥ 50 µm in length were seen that had either near-normal levels of glutamate (Mild, High Glu) or decreased levels of glutamate (Mild, Low Glu). (b) The glutamate immunostaining density in the Müller cell process of the nerve fiber layer and putative Müller cell bodies of the INL was increased in PG compared to control. Bars = SEM. *$P < 0.05$ by Tukey's multiple comparison test.

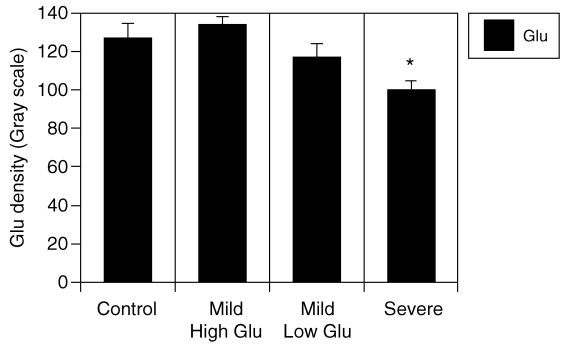

Figure 3. Glutamate immunoreactivity was maintained at high levels in the endothelium of blood vessels in PG. Replicate sections from control and PG eyes were immunogold stained at the same time and images captured under the same conditions. Bars = SEM. *$P < 0.05$ by Tukey's multiple comparison test compared to control.

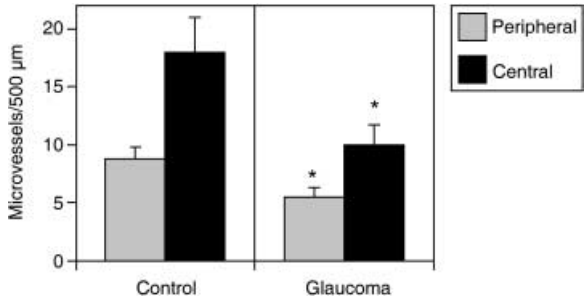

Figure 6. Microvessel densities were reduced in the retinas of dogs with PG when either lectin staining or laminin immunohistochemistry was used to identify vessels. The number of microvessels in 500-µm fields of paraffin sections were counted in the peripheral retina (starting 1 mm from the ora ciliaris) and central retina in sagittal sections of eyes stained with either lectin or antibodies to laminin. Three fields were counted from each region of each retina. *$P < 0.05$ by Tukey's multiple comparison test.

of microvessels with lectin and antibodies to laminin is illustrated in Fig. 5.

Microvessel densities in both the peripheral and central regions of retinas were reduced in PG retinas (Fig. 6). The

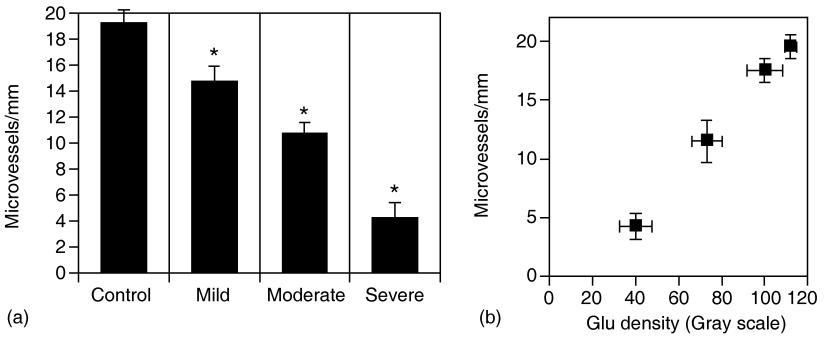

Figure 4. Microvessels were lost in regions with increased glutamate redistributon and increased severity of damage. (a) Microvessels were counted in 200-µm regions of retina with different severities of damage in the INL. Microvessels were identified by the high levels of glutamate retained in endothelial cells. *$P < 0.05$ by Tukey's multiple comparison test compared to control. (b) Microvessel densities from regions with different severities of damage were plotted against the average glutamate staining density in the INL of the same regions. Glu densities are those from Fig. 2a. Bars = SEM.

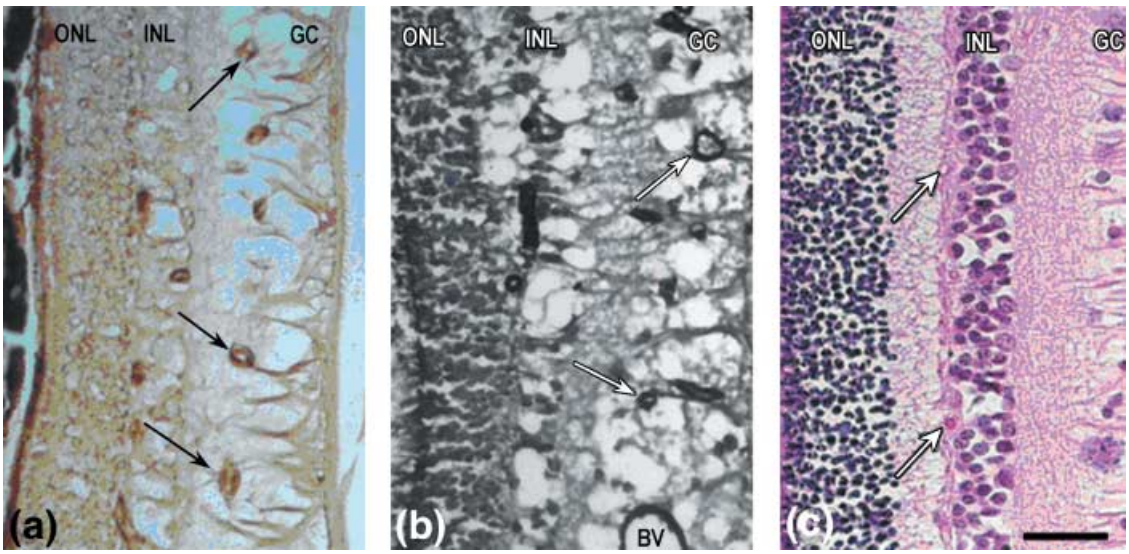

Figure 5. Staining of microvessels in paraffin sections with lectin and antibodies to laminin. (a) A section of control retina immunohistochemically stained for laminin shows labeling of microvessels, three of which are indicated by arrows. GC indicates the ganglion cell layer. (b) A section from a PG retina stained with lectin shows labeling of microvessels, two of which are indicated by arrows. BV indicates a larger blood vessel. (c) A section of control retina stained with hematoxylin and eosin illustrates the appearance of typical microvessels (arrows). All images are shown with the same orientation and magnification. Bars = 25 µm.

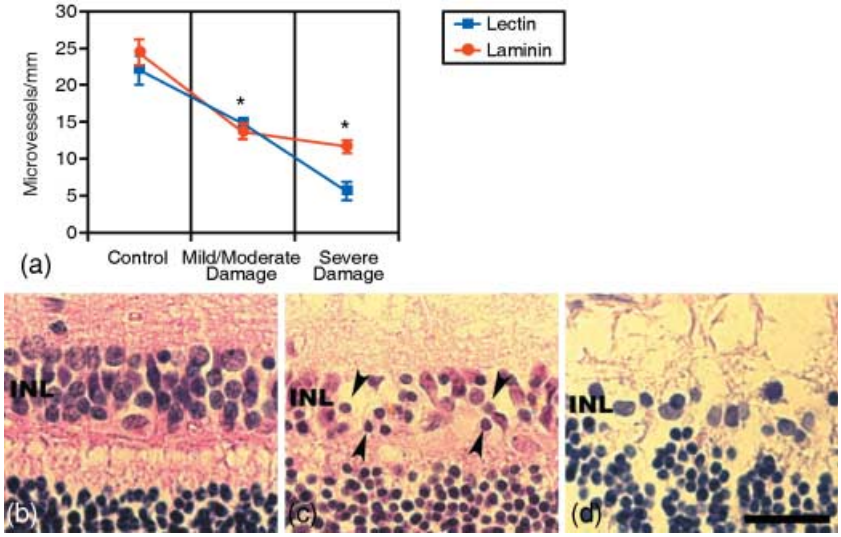

Figure 7. (a) Microvessel densities were decreased in regions with increased severity of damage when either lectin staining or laminin immunohistochemistry were used to identify blood vessels. Microvessels were counted in 250-μm regions of retina with different severities of damage in the INL. Bars = SEM. *$P < 0.05$ for both lectin and laminin compared to control (Tukey's multiple comparison test). (b) Control retina showing lack of damage to the INL. (c) Moderately damaged retina showing damaged INL cells (arrows). (d) Severely damaged retina with reduced INL thickness. (b–d) Hematoxylin and eosin staining. All images are shown with the same orientation and magnification. Bar = 25 μm.

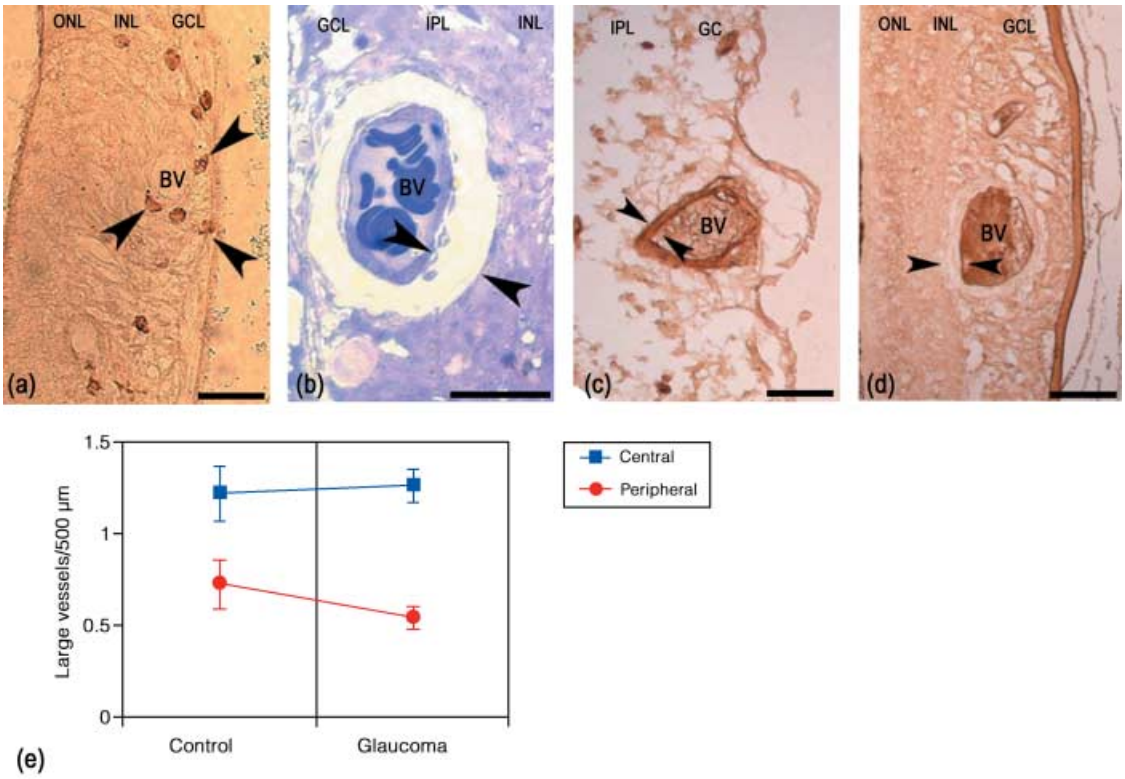

Figure 8. Larger vessels showed signs of inflammation and damage in PG retinas. (a) Immunohistochemical staining for CD3 revealed vascular cuffing with T lymphocytes around some vessels (arrowheads) of PG retinas. Bar = 12 μm. BV, blood vessel. BV indicates a blood vessel. GCL indicates the ganglion cell layer. (b) Larger vessel walls were often thickened in PG. Arrowheads indicate the thickened wall of a blood vessel. IPL indicates the inner plexiform layer. Plastic section stained with toluidine blue. Bar = 10 μm. (c) Albumin was often found outside the lumen of blood vessels in PG. Immunohistochemical staining for albumin was especially prominent in the walls of blood vessels (arrowheads). Bar = 12 μm. (d) Albumin was normally found only in the lumen of vessels in control retinas immunohistochemically stained for albumin. Arrowheads indicate the unlabeled wall of a blood vessel. Bar = 12 μm. (e) No significant loss of larger vessels was apparent in PG. Bars = SEM.

number of microvessels in 500-μm fields of sections of control and PG retinas stained with lectin and antibodies to laminin was counted. Significant decreases in microvessel densities were seen in both central and peripheral regions of PG eyes.

The densities of microvessels in regions with different degrees of damage were quantified using lectin staining and laminin immunohistochemistry to identify blood vessels (Fig. 7). The density of microvessels was reduced as the severity of damage increased in a manner similar to that

seen when glutamate was used as a marker for endothelial cells in plastic sections (Fig. 4b).

Larger vessels were damaged but not lost in PG

In contrast to microvessels, the density of larger vessels (lumen > 10 μm) was not significantly reduced in PG (Fig. 8a). However, the larger vessels showed signs of damage, including thickened vessel walls (Fig. 8b), albumin leakage (Fig. 8c,d) and cuffing by leukocytes (Fig. 8e). The finding of albumin immunoreactivity outside the lumen of larger vessels in PG suggests a breakdown of the blood–retinal barrier.

Microvessel obliteration in PG does not require the loss of photoreceptors

Microvessel obliteration occurs in several retinal diseases, including those where hyperoxia is induced by a loss of photoreceptors. To determine if photoreceptor loss was necessary to induce microvessel obliteration, we measured the thickness of the ONL in the central region of chronic PG retinas where microvessel loss occurs (Fig. 6). No decrease in ONL thickness was seen in chronic PG retinas compared to control retinas. The central ONL thickness was 33 mm ± 3.6 in control retinas and 43.8 mm ± 4.4 in chronic PG ($P > 0.05$). This suggests that thinning of the outer retina with a subsequent hyperoxia is not necessary to induce microvessel obliteration.

DISCUSSION

In previous studies of PG in dogs we reported that alterations in glutamate distribution occurred in regions with more neuronal damage. This glutamate redistribution was consistent with an ischemia-induced glutamate release that we hypothesized may have contributed to the damage.[7,11] In other studies, vascular abnormalities have been reported in canine glaucoma,[9,10] including PG.[8] In the current study we tested the hypothesis that vascular damage may lead to microvessel obliteration and focal ischemia. We compared the microvessel densities of retinal regions with differing degrees of damage and glutamate redistribution to determine if microvessel loss may contribute to glutamate redistribution and retinal damage in PG. We also examined the types of damage seen in larger vessels of PG retinas to better understand the pathogenesis of the disease and to determine if leakage of glutamate from vessels might contribute to the pathologic glutamate redistribution previously reported in PG.[7,11]

A loss of microvessels and damage to larger vessels may occur in PG retinas

In this study we found that the densities of microvessels in PG eyes were significantly reduced compared to control retinas. This finding was consistent in all three of the different methods of identifying blood vessels (Figs. 4,7). In contrast to the microvessels, the densities of larger vessels in both the central and the peripheral regions of PG retinas were not significantly reduced (Fig. 8e). This suggests that a selective loss of microvessels occurs as PG progresses. This loss could be similar to diseases such as microscopic polyangitis, in which small vessels, but not larger vessels, develop vasculitis.[20,21]

The loss of microvessels appears greatest in the most severely damaged retinal regions. Neuronal damage in PG is characterized by swelling of cell bodies and nuclear changes in the INL. This is especially prominent in acute PG.[7] In some regions of the retina the INL and ONL may be thinned[6,7] as a result of neuronal loss that presumably follows the neuronal damage. This study supports the idea that decreases in microvessel numbers are dependent on the severity of INL damage, which suggests that the loss of microvessels may increase as retinal damage progresses.

In a concurrent study we found vascular changes in the larger blood vessels of retinas of glaucomatous dog eyes that were consistent with inflammation. These changes include an infiltration of leukocytes around some larger vessels, thickening of vessel walls, and increased permeability of the blood vessels to albumin (Fig. 9) and possibly to other smaller molecules such as glutamate.

Glutamate leakage from damaged vessels into the extracellular fluid of the retina potentially contributes to ongoing retinal damage. It is well established that high extracellular levels of glutamate are toxic to retinal neurons.[22,23] Plasma concentrations of glutamate are variable but have typically been reported to be about 50 μM.[24] The extracellular fluid of the central nervous system, including the normal retina, is believed to contain only very low levels of glutamate, perhaps only a few μM, as seen in normal vitreous of dogs.[25] This suggests that glutamate will leak from the blood into the retina down a concentration gradient. The initial exposure of the retina to higher levels of glutamate may not cause neuronal damage, owing to the very robust uptake systems for glutamate present on glial cells. However, it is likely that in regions where the glial cells may be low on energy, glutamate uptake and conversion to glutamine will be reduced. Both the uptake of glutamate and formation of glutamine are energy-requiring processes and may be reduced in ischemic regions, perhaps including regions of microvessel loss.

Glutamate redistribution may occur in mildly damaged regions and is associated with a loss of microvessels

The relationship between glutamate redistribution and microvessel loss and neuronal damage was examined in plastic-embedded eyes. Glutamate redistribution could be detected in these sections, even in some mildly damaged regions of INL (Fig. 2). The regions with glutamate redistribution also had significantly lower microvessel densities (Fig. 4b). This is consistent with the idea that vascular changes initiate early glutamate redistribution and PG damage. However, it should be noted that both damage and glutamate redistribution were observed in cells very close to remaining microvessels. This indicates that ischemia induced by microvessel loss is not the sole factor involved in inducing glutamate redistribution or neuronal damage.

Mechanisms that may induce microvessel loss

In some retinal diseases, such as retinopathy of prematurity,[12] retinal degeneration[15] and retinitis pigmentosa,[14] microvessel loss is believed to occur in response to hyperoxia. The hyperoxia may be due to decreased oxygen demand induced by a previous loss of retinal cells. For example, in retinal degenerations in which photoreceptors are lost, microvessel obliteration has been induced in the retina that is believed to be secondary to increased oxygen availability from the choroid vasculature.[14]

To determine if photoreceptor loss might account for the microvessel loss in PG, we measured the thickness of the ONL in the central portion of chronic PG retinas where microvessel obliteration occurred. No thinning of the ONL was seen in these regions of microvessel obliteration, suggesting that photoreceptor loss, decreased oxygen demand, and a subsequent hyperoxia was not the mechanism leading to microvessel obliteration in PG. However, in PG there is a greater loss of microvessels in regions of severe damage, where thinning of the INL has occurred. This suggests that loss of neurons of the inner retina may lead to decreased oxygen demand, and a subsequent hyperoxia that may induce a subsequent pruning of microvessels. However, the observation that some of the microvessel loss occurs in mildly damaged INL prior to substantial thinning of the INL supports the argument that hyperoxia due to thinning of the retina does not initiate microvessel loss in PG.

Implications for future studies and treatment

Losses of microvessels and glutamate redistribution may occur even in mildly damaged regions in PG retinas. If these processes contribute to early PG damage, glutamate antagonists may prove effective in reducing or preventing early excitotoxic damage to neurons. However, the mechanism(s) that induce microvessel loss and damage to larger blood vessels remain to be determined. Without effective treatments for the vascular changes, these changes may continue to contribute to the progressive retinal damage seen in PG.

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