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Anterior optic nerve microvascular changes in human glaucomatous optic neuropathy

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Abstract

The microvascular changes in the anterior optic nerve in human glaucomatous eyes were examined by selective methylmethacrylate microvascular corrosion castings following cannulation of the central retinal artery and posterior ciliary arteries in 11 normal eyes and 9 glaucomatous eyes. The resulting castings were examined with scanning electron microscopy. Microvascular changes were found in the anterior optic nerves of all the glaucomatous eyes with visual function loss. These findings include areas of capillary filling defects within the anterior optic nerve and a decreased numbers of feeding arteriolar vessels to the anterior optic nerve. In the prelaminar and laminar regions, the typical capillary patterns are lost and laminar striations are not present. Juxtapapillary choroidal and retinal avascular areas were also identified in two of the glaucomatous eyes. Selective microvascular corrosion casting is an excellent method to examine the three-dimensional microvasculature of the anterior optic nerve. Microvascular changes in the anterior optic nerve may play a role in the development of glaucomatous optic neuropathy.

Key words Glaucoma, Microcirculation, Optic nerve

The pathogenesis of glaucomatous optic neuropathy has been studied extensively and regional microvascular changes have been cited as a potential contributor. The microvasculature of the anterior optic nerve in glaucoma has been the subject of histological, clinical and experimental investigations. Many studies have suggested that vascular factors can contribute to glaucomatous optic neuropathy.[1–6]

A variety of investigational techniques have been developed to examine the anterior optic nerve microvasculature and blood flow, both *in vitro* and *in vivo*.[2–11] Selective microvascular corrosion casting techniques are an established method to study the three-dimensional vascular patterns of the anterior optic nerve. The microvasculature of the human anterior optic nerve in normal and glaucomatous eyes has been described, using selective cannulation methods and methylmethacrylate.[2,7] The purpose of the current study was to investigate the microvascular casting changes of the anterior optic nerve in the human glaucomatous eye.

Materials and methods

Human eye bank eyes, with at least 10 mm long retrobulbar optic nerves (allowing identification of the central retinal artery and the posterior ciliary arteries), were obtained within 24 h of death. All procedures followed the tenets of the Declaration of Helsinki.

Eleven normal eyes were obtained from 6 Caucasian subjects ranging from 63 to 83 years of age (mean age 74.9 ± 7.9 years) which were 20.5 ± 8.7 h (average time after death) post-mortem. Nine glaucomatous eyes were obtained from 5 subjects 70 to 88 years of age (mean age 78.4 ± 7.5 years) which were 20.9 ± 6.3 hours post-mortem. No pre-mortem systemic vascular disorders or diabetes were found in the normal subjects.

Using a previously described method to selectively cannulate the posterior ciliary arteries and central retinal arteries vascular corrosion castings were developed.[2,7,12] Pulled polyethylene tubing attached to a 21 gauge needle with tapered tip and MicroFil tubes (World Precision Instruments, Sarasota, FL) were used to cannulate the central retinal artery and posterior ciliary arteries. The tubings were ligated in place by 7-0 or 9-0 nylon sutures. Tissue plasminogen activator (Activase, Genetech, South San Francisco, CA) was injected to flush the microvessels. After flushing four times with tissue plasminogen activator, modified Batson's No. 17 methylmethacrylate medium[12] (Polysciences, Warrington, PA), the viscosity of which is about 11 cPs, was manually injected by 1 ml plastic syringe until the plastic material was observed outflowing from the

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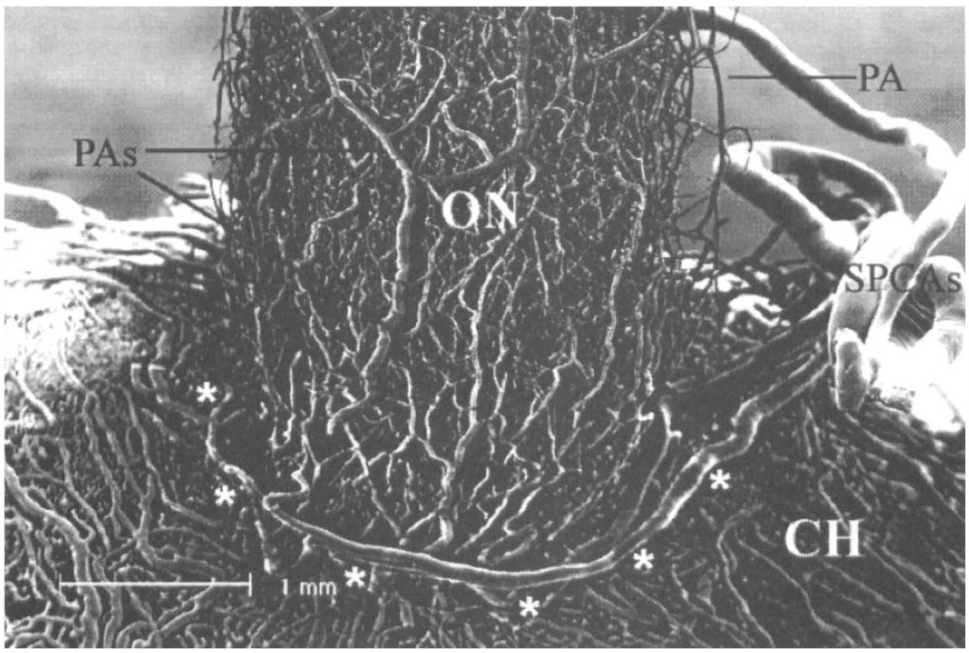

Fig. 1. *Methylmethacrylate vascular casting of the right eye of an 83-year-old male. Posterior view shows complete vascular filling of the circle of Zinn-Haller (asterisks) and the anterior optic nerve (ON) as the ON enters the posterior aspect of the eye. Note the multiple 'feeding vessels' branching from the circle of Zinn-Haller and supplying the anterior optic nerve. SPCAs, short posterior ciliary arteries; PAs, pial arteries; CH, choroid.*

vortex veins and/or central retinal vein. Two hours later, the eyes were immersed in 10% buffered formalin for 24 h to allow complete polymerisation. All the surrounding tissue was corroded in 6 M potassium hydroxide (45–50 °C for 24–48 h), and the vascular castings were rinsed with running water and then air-dried completely at room temperature.

Under a binocular dissecting microscope, each casting was oriented and the blood supply to the anterior optic nerve identified. Microdissection was carefully performed to remove the extravasation and to show the detail of the anterior optic nerve. Microdissected castings were mounted on aluminium stubs, sputter-coated with gold–palladium (30 nm), and examined under a working accelerated voltage of 2 kV, in a scanning electron microscope of total accelerated voltage 30 kV (Philips XL30, The Netherlands). Imaging photographs were taken.

Results

Normal microvasculature of the anterior optic nerve

Among the 11 normal eyes, the anatomy of the microvasculature was similar to our previous studies with normal human eyes.[7] The microvasculature of the normal human anterior optic nerve clearly demonstrated complete microvascular filling of the anterior optic nerve and the circle of Zinn-Haller (Fig. 1). The anterior optic nerve can be divided into four anatomical regions: the superficial nerve fibre layer, the prelaminar region, the lamina cribrosa and the retrolaminar region (Fig. 2). The superficial nerve fibre layer is supplied from branches of the central retinal arteries. Branches either from the circle of Zinn-Haller or from the short posterior ciliary arteries provide the primary blood supply to the prelaminar and laminar regions. In the prelaminar and lamina cribrosa regions, most of the capillaries run parallel to the posterior sclera. The capillaries in these regions are within the connective tissue plates and therefore have a striated appearance. In the normal casting specimens, we found the number of feeding arteriolar vessels supplying the anterior optic nerve (prelaminar and laminar regions) to be 9.6 ± 1.5 (mean ± SD) and 10.8 ±1.9 in the superior and inferior quadrants of the anterior optic nerve, respectively (Table 1). The retrolaminar region is supplied by branches of the short posterior ciliary arteries and pial arteries, as well as occasional branches of the central retinal artery.

Table 1. *The number of feeding arterioles from short posterior ciliary arteries and the circle of Zinn-Haller to the anterior optic nerve in normal eyes (n = 5)*

	No. of vessels	
	Superior quadrant	Inferior quadrant
Normal eyes	9.6 ± 1.5	10.8 ± 1.9

Values are the mean ± SD.

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Table 2 illustrates the cannulation sites and pre-mortem clinical data for the 9 glaucomatous eyes. The age of the patients ranged from 70 to 88 years (mean ages 78 ± 6.5) with a history of glaucoma of 7–19 years. Seven of 9 eyes had pre-mortem visual function changes consistent with glaucoma. Both the medial and lateral posterior ciliary arteries and central retinal artery were cannulated in 8 of 9 eyes. The central retinal artery could not be identified

Table 2. *Cannulation sites and clinical data of the glaucomatous eyes*

Age (years)	Sex	Diagnosis	Eye	Cannulation	Max. IOP (mmHg)	C/D ratio	Visual fields
70	M	POAG	OD	PCAs	40	0.6	Inferior arcuate
70	M	POAG	OS	CRA + PCAs	40	0.7	Inferior arcuate
74	M	POAG	OD	CRA + PCAs	38	0.9	Severe visual loss
74	M	POAG	OS	CRA + PCAs	23	0.5	WNL
83	F	POAG	OD	CRA + PCAs	23	0.6	WNL
83	F	POAG	OS	CRA + PCAs	36	0.7	Scotoma
81	F	POAG	OD	CRA + PCAs	30	> 0.95	Blind
81	F	POAG	OS	CRA + PCAs	30	0.9	Constriction
88	M	POAG	OS	CRA + PCAs	36	0.7	Superior and inferior

POAG, primary open-angle glaucoma; OD, rigid eye; OS, left eye; PCAs, posterior ciliary arteries; CRA, central retinal artery; C/D ratio, cup/disc ratio; WNL, within normal limitation.

in 1 eye. All the eyes with visual function loss demonstrated microvascular changes. There were three principal findings in the glaucomatous eyes:

1. The anterior optic nerves had areas of capillary loss and fewer feeding arteriolar vessels (range 3–6) in all eyes with visual function defects (Fig. 3).
2. In the prelaminar and laminar regions, the typical capillary patterns or striations were lost (Fig. 2). Capillary dropout mainly occurred within the prelaminar and laminar regions of the anterior optic nerve.
3. Juxtapapillary choroidal and retinal avascular areas were found in 2 of the glaucomatous eyes.

Discussion

Selective vascular casting in normal and glaucomatous anterior optic nerve provides three-dimensional models of the microvasculature of the anterior optic nerve. Anterior optic nerve microvascular changes have been demonstrated in primary open-angle glaucoma in a variety of studies.[2–5,13,14]

Previous studies have suggested an *in vivo* reduction of blood flow in the juxtapapillary retina and neuroretinal rim area of the glaucomatous optic nerve head, and a reduction in the neuroretinal rim blood flow that is proportional to the increase in the cup/disc ratio.[3] In another study using retinal flowmetry, 63% of glaucoma patients had avascular areas in the lamina cribrosa.[4] This finding suggests that glaucoma patients tend to have smaller blood volume, flow and velocity in the lamina cribrosa. Hayreh[14] found a reduction in fluorescence of the optic disc in a large number of glaucoma patients with significant changes at the optic disc and visual field defects by using fluorescence angiography. Clinical observations also support the theory that arterial insufficiency and decreased blood flow in the anterior portions of the optic nerve are potential factors in the development of visual field loss in glaucoma.[15]

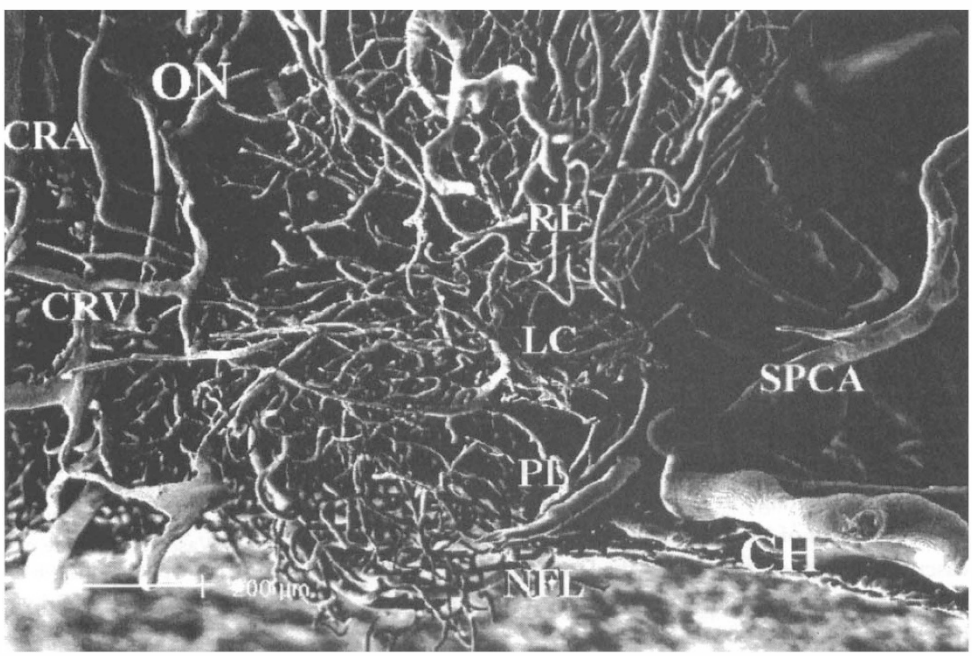

Fig. 2. *Posterior view of a microdissected vascular casting of the anterior optic nerve from a 76-year-old female, demonstrating the anatomical regions and blood vessels of the hemisection of optic nerve. NFL, nerve fibre layer; PL, prelaminar region; LC, laminar cribrosa; RL, retrolaminar region; ON, optic nerve; CRA, central retinal artery; CRV, central retinal vein; SPCAs, short posterior ciliary artery; CH, choroid.*

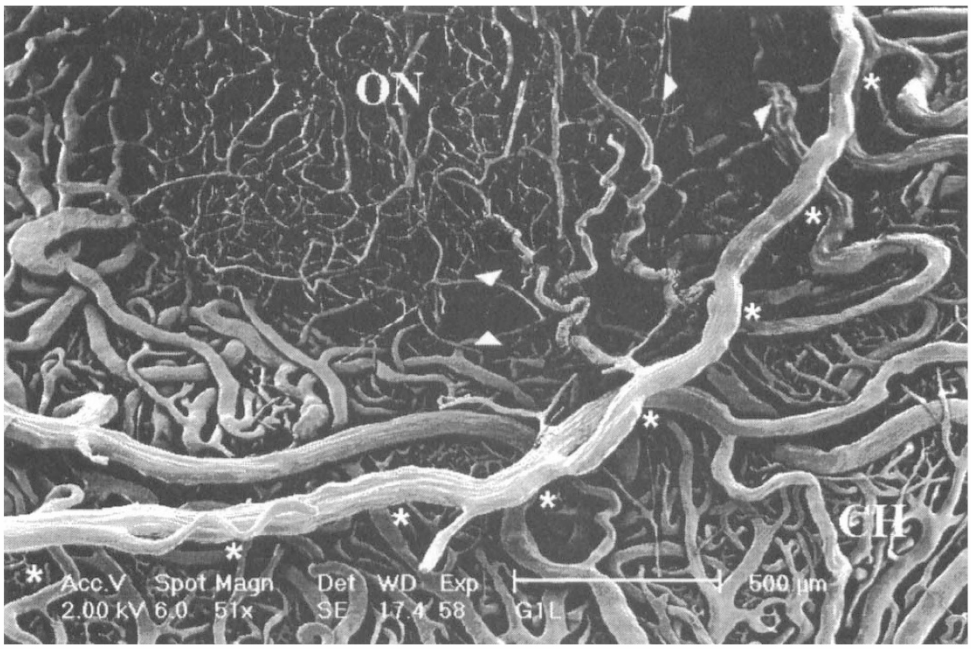

FIg. 3. *Vascular casting of the anterior optic nerve from a 70-year-old male, with a glaucoma history of 19 years in the left eye. This view shows an irregular area of a capillary filling defect (within the arrowheads) from the superior temporal to superior nasal aspect. Also note the loss of feeding vessels from the circle of Zinn-Haller in the same region. Asterisks, the circle of Zinn-Haller; CH, choroid; ON, optic nerve.*

In experimental glaucoma, François and Neetens[15] reported that there was significant reduction in the filling of the capillaries of the retina and choroid, and in the optic nerve the reduction was greater on the temporal side. This suggests that an alteration in the blood flow associated with increased intraocular pressure may be a pathological change in the optic nerve.[5,16] In addition, Hayreh and colleagues[17] found that 70% of the choriocapillaris and 67% of the temporal peripapillary choroid showed moderate to severe atrophy.

Quigley and his co-workers[18] suggested that the loss of nerve fibres within the anterior optic nerve leads to capillary loss and that a constant relationship between the tissue and capillary ratio was maintained after complete transection of optic nerve. This was also seen in human glaucomatous eyes.[13]

In the current study, microvascular changes were found in the anterior optic nerve and juxtapapillary choroid and retina in the glaucomatous eyes. This suggested that ischaemic changes may be associated with the development of glaucomatous optic neuropathy. However, these post-mortem anatomical observations can not determine whether vascular changes lead to the neuropathy or the neuropathy leads to the vascular changes. It is important to provide sufficient blood flow to the anterior optic nerve so that normal visual function can be maintained. As the damage in glaucoma is believed to occur at the level of the lamina cribrosa,[19,20] the finding of vascular anatomical changes at the laminar level further supports the ischaemia-induced neuropathy theory. Capillaries may be altered or collapsed by compressive changes of the laminar cribrosa. The loss of typical striated patterns within the anterior optic nerve may be associated with the changes in the laminar plates during the development of glaucomatous optic nerve neuropathy. However, a causal relationship remains to be proven.

To our knowledge, the present study is the first to employ vascular casting techniques in human glaucomatous eyes to illustrate the three-dimensional microvascular changes associated with glaucomatous optic neuropathy. Microvascular changes were found in the anterior optic nerve and also the juxtapapillary choroid and retina of the glaucomatous eyes. It is possible that haemodynamic changes resulting from anatomical microvascular changes of the anterior optic nerve play an important role in the pathogenesis of glaucomatous optic neuropathy.

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