Inter-Relationship of Arterial Supply to Human Retina, Choroid, and Optic Nerve Head Using Micro Perfusion and Labeling

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PURPOSE. The prevailing view is that the human retina is supplied by the central retinal artery (CRA), the short posterior ciliary arteries (SPCAs) support the choroid, and both the CRA and the SPCAs are so-called “end artery” systems. In this study, we investigate whether vascular connections among the retina, choroid, and the optic nerve head (ONH) exist, using selective cannulation and microperfusion-labeling techniques.

METHODS. The CRA and/or one or more of the SPCAs were selected for cannulation in 18 human donor eyes. Fluorescent probes with different excitation wavelengths were perfused through different arteries on the same eye to distinguish the supply sources of different vascular beds. After labeling and fixation, the ONH region was dissected either longitudinally or transversely as thick sections for confocal microscopy. Retina, choroid, and ONH were imaged from whole-mount specimens.

RESULTS. Probes perfused through the CRA or the SPCA alone labeled the microvessels in the retina, choroid, and ONH regions, as well as the optic nerve trunk. The vessels of the lamina cribrosa and the optic nerve trunk were labeled when probes were perfused through the SPCA. Perfusion through both the CRA and SPCA produced double labeling of vessels in the retina, the choroid, and the ONH.

CONCLUSIONS. The results indicate an inter-relationship of arterial supply to the retina, choroid, and ONH in the human eye. This has important implications in understanding clinical observations and disease mechanisms such as that of glaucoma and ischemic optic nerve disease.

Keywords: retinal vasculature, choroidal vasculature, optic nerve head vasculature

The retinal and choroidal circulations have been described as “end-arterial” systems without anastomoses.1,2 The retinal circulation is responsible for feeding a high metabolic rate tissue mainly the inner retina.3,4 The choroid primarily supplies the outer retina. The optic nerve head (ONH) is a unique region where approximately 1.2 million axons from the retinal ganglion cells converge to exit from the globe. This region has high metabolic demand as reflected by the regional confinement of cytochrome oxidase enzyme and the presence of a dense capillary network.5–7 The efficient supply of nutrients and oxygen and efficient removal of wastes from retina and ONH are therefore crucial for the survival and function of the axons.

Ischemic disorders of the ONH such as anterior ischemic optic neuropathy are recognized as common sight-threatening diseases.8 It also is clearly evidenced that localized insufficiency of the ONH blood supply is closely correlated with visual field defects and nerve fiber layer loss in glaucoma patients.9–11 This hemodynamic alteration has been proposed to play an important role in the pathogenesis of glaucomatous optic neuropathy by increasing the susceptibility of the ONH to elevated intraocular pressure.12 To understand the potential role of vascular risk factors in glaucoma and ischemic disorders of the ONH and retina, it is important to understand their vascular supply anatomically and functionally.

Anatomically arterial pathways to the ONH, choroid, and retina are derived from the posterior ciliary arteries (PCAs) and central retinal artery (CRA) branches of the ophthalmic artery (OA). The CRA primarily supplies the retina, whereas short PCAs (SPCAs) segmentally supply the choroid. Evidence from previous studies of vasculature in the ONH have demonstrated that both CRA and SPCAs have branches that supply the ONH,13–17 although the extent of cross-communication is unknown.

In this study, we did not attempt to investigate the detailed arterial pathways that have remained controversial for decades and have interindividual variations.18 The question we were interested in is whether these seemingly separate arterial sources to the highly metabolically active regions of ONH, choroid, and retina have the potential to interact and collaborate downstream. Such an inter-relationship may help us to better understand the blood supply to the ONH, choroid, and retina.

To explore the inter-relationship between major arterial pathways of the PCAs, we used selective cannulation of either the CRA and/or the SPCA and perfusion with various wavelength labels in human donor eyes.3,19,20 The arterial downstream distributions were determined by different labeled vasculature in the ONH, retina, and choroid.
TABLE 1.  Details of Human Donor Eyes Studied

<table>
<thead>
<tr>
<th>Eye ID</th>
<th>Age (y) and Sex</th>
<th>COD</th>
<th>PMT (h)</th>
<th>Cannulation Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>59 M</td>
<td>Stroke</td>
<td>17</td>
<td>CRA-lectins FITC</td>
</tr>
<tr>
<td>B</td>
<td>65 M</td>
<td>Liver cirrhosis</td>
<td>18</td>
<td>CRA-VEC/eNOS/f-actin</td>
</tr>
<tr>
<td>C</td>
<td>65 M</td>
<td>Liver cirrhosis</td>
<td>18</td>
<td>CRA-VEC/eNOS/F-actin</td>
</tr>
<tr>
<td>D</td>
<td>62 F</td>
<td>SAH</td>
<td>6</td>
<td>CRA-ConA/Occludin-FITC; PCA-Actin</td>
</tr>
<tr>
<td>E</td>
<td>64 F</td>
<td>Cardiac arrest</td>
<td>12</td>
<td>CRA-asSMA/VEC/Cpn; tLPCA–PY20/VEC/asSMA</td>
</tr>
<tr>
<td>F</td>
<td>31 M</td>
<td>Suicide</td>
<td>6</td>
<td>CRA-Actin TRITC; tSPCA–Actin FITC</td>
</tr>
<tr>
<td>G</td>
<td>31 M</td>
<td>Suicide</td>
<td>6</td>
<td>CRA-Actin TRITC; tSPCA–Actin FITC</td>
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<tr>
<td>H</td>
<td>58 F</td>
<td>Suicide</td>
<td>Unknown</td>
<td>CRA-Actin TRITC; tSPCA lectin FITC</td>
</tr>
<tr>
<td>I</td>
<td>38 F</td>
<td>Suicide</td>
<td>Unknown</td>
<td>CRA-lectin TRITC; tSPCA-lectin FITC</td>
</tr>
<tr>
<td>J</td>
<td>67 M</td>
<td>Cirrhosis and renal failure</td>
<td>22</td>
<td>CRA-VEC/eNOS/ActinAF647; tSPCA1–Actin FITC; tSPCA2 (LPCA temp branch)–Actin TRITC</td>
</tr>
<tr>
<td>K</td>
<td>60 M</td>
<td>Cancer</td>
<td>12</td>
<td>CRA-VEC/ActinAF647; tSPCA1–Actin FITC; tSPCA2 (LPCA temp branch)–Actin TRITC</td>
</tr>
<tr>
<td>L</td>
<td>22 M</td>
<td>Suicide</td>
<td>15</td>
<td>CRA-Actin AF546; tPCA–Actin AF635</td>
</tr>
<tr>
<td>M</td>
<td>68 F</td>
<td>Cancer</td>
<td>11</td>
<td>tSPCA–Actin FITC</td>
</tr>
<tr>
<td>N</td>
<td>68 F</td>
<td>Cancer</td>
<td>11</td>
<td>tSPCA–Actin TRITC; nSPCA–Actin FITC</td>
</tr>
<tr>
<td>O</td>
<td>43 M</td>
<td>Unknown</td>
<td>10</td>
<td>tSPCA–Actin FITC; tLPCA–Actin AF647</td>
</tr>
<tr>
<td>P</td>
<td>25 M</td>
<td>Pneumonia</td>
<td>18</td>
<td>2 × tSPCA–VEC/Cpn/Actin AF647</td>
</tr>
<tr>
<td>Q</td>
<td>25 M</td>
<td>Pneumonia</td>
<td>18</td>
<td>tSPCA–VEC/Cpn/Actin AF647</td>
</tr>
<tr>
<td>R</td>
<td>61 M</td>
<td>Cardiac</td>
<td>12</td>
<td>2 × tSPCA–Actin FITC/Actin TRITC</td>
</tr>
</tbody>
</table>

AF, Alexa Fluor; COD, cause of death; ConA, Concavalin A; eNOS, endothelial nitric oxide synthase; PMT, postmortem time; PY20, phosphotyrosine.

MATERIALS AND METHODS

This study was approved by the human research ethics committee at the University of Western Australia. All human tissue was handled according to the tenets of the Declaration of Helsinki.

Human Donor Eyes

A total of 18 human eyes from 13 postmortem donors were used. All eyes were obtained from the Lions Eye Bank of Western Australia with valid consent for use of tissue for research purposes. All the eyes were received after the removal of corneal buttons for transplantation. None of the donors had a known history of glaucoma, ischemic optic neuropathy, CRA, or central retinal vein (CRV) occlusion. The demographic data, cause of death, postmortem time to eye perfusion, and the probes applied to each donor eye are listed in Table 1.

Micro-Cannulation

Micropipettes made from glass tubes with an outer diameter of 120 to 180 μm were used to cannulate selected arteries at the back of the globe (Fig. 1). The origin of the CRA is variable (from OA or PCA); however, the point of penetration into the dural sheath was a consistent finding (penetrating the optic dural sheath at its inferior side approximately 8 to 10 mm behind the globe).21 Each main PCA divides into approximately 10 to 20 SPCAs just before, or after, penetrating the sclera. Three eyes were cannulated only at the CRA before it dives into the optic nerve. Seven eyes were cannulated at the CRA and one SPCA close to the ONH on the temporal side. Two eyes were cannulated at the CRA and two SPCAs close to the ONH on the temporal side. Six eyes had one or two SPCAs cannulated but not the CRA.

Intravascular Perfusion and Fluorescent Probes Labeling

After cannulation, the artery was perfused with 1% BSA Ringers for 20 minutes to wash out any remaining blood cells from the region supplied by the cannulated artery. Perfusion pressure was monitored throughout and flow rate adjusted (between 50 and 100 μL/min) to ensure the perfusion pressure did not exceed 70 mm Hg. This was followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer for 20 minutes. The steps that followed varied depending on the fluorescent probe used and are detailed in separate paragraphs below. Phalloidin was the main molecule used as it binds to cytoskeleton F-actin, which is known to be abundantly present in all cells and allows the distinction between arterioles and venules based on the F-actin and smooth muscle distribution pattern. Lectin was also used to label glycolcalyx on the intraluminal side of endothelium. Lectin has the advantage of giving more precise labeling as it generally does not leech into the extraluminal region. Antibodies against endothelial and smooth muscle markers were used on some eyes, mostly in conjunction with phalloidin, to label for junctional protein distribution and for potential smooth muscle contractility.

Four of the eyes were then perfusion labeled over the course of 2 hours using 1 mL 0.05 mM lectin from *Triticum vulgaris* conjugated with FITC (Sigma L4895; Sigma-Aldrich Pty. Ltd., St. Louis, MO, USA) or tetramethylrhodamine (TRITC; Sigma L5266). Any remaining lectin was washed out from the intraluminal side of endothelium. Lectin has the advantage of giving more precise labeling as it generally does not leech into the extraluminal region. Antibodies against endothelial and smooth muscle markers were used on some eyes, mostly in conjunction with phalloidin, to label for junctional protein distribution and for potential smooth muscle contractility.

Eight eyes were perfused with 0.1% Triton X-100 in 0.1 M phosphate buffer for 5 to 7 minutes to induce increased permeability for phalloidin. The residue Triton X-100 was washed out by continuous perfusion of phosphate buffer for another 20 minutes; 0.001 M phalloidin conjugated to FITC (Sigma P5282) or TRITC (Sigma P1951) was perfused.
separately through the respective arteries over the course of 2 hours to allow ample time for sufficient labeling. Residue phalloidin was washed out by perfusing phosphate buffer for a further 30 minutes. The globes were then immersion-fixed in 4% PFA overnight in the refrigerator protected from light.

Six eyes were perfusion-labeled using the indirect immunofluorescent protocol. After the 4% PFA fixation step, residue fixatives were washed out from the system by continuous perfusion of 0.1 M phosphate buffer for 20 to 30 minutes. A solution comprising 10% donkey serum, 0.1% Triton X-100, and primary antibodies (1 in 50 dilution) was gradually injected into the cannula over the course of 1.5 hours. Residual primary antibodies were then removed by continuous perfusion of phosphate buffer for 30 minutes. This was followed by gradual injection of the corresponding secondary antibodies tagged with fluorescent probes, together with or without 30 U phalloidin conjugated to Alexa Fluor 647 or 488 (A22287 and A12379; Thermo Fisher Scientific, MA, USA) over the course of 1 hour. Residual fluorescent tagged secondary antibodies and phalloidin were then washed out by continuous perfusion of phosphate buffer for 30 minutes. The globes were then immerse-fixed overnight in 4% PFA in the refrigerator protected from light.

Primary antibodies used included goat anti-VE-Cadherin (SC-6458, six eyes; Thermo Fisher Scientific), rabbit anti-eNOS (ab66127, three eyes; Abcam, Melbourne, Victoria, Australia), rabbit anti-occludin (ab31721, one eye; Abcam), goat anti-aSMA (ab21027, one eye; Abcam), rabbit anti-calponin (ab46794, three eyes; Abcam), rabbit anti-zSMA (ab5694, one eye; Abcam), and rabbit anti-PTYR731 (SAB 4504676, one eye; Sigma-Aldrich Pty. Ltd.).

Secondary antibodies used included donkey anti-goat conjugated with Alexa Fluor 555 (Abcam ab150130), donkey anti-rabbit conjugated with Alexa Fluor 488 (Invitrogen A21206), and donkey anti-mouse conjugated with CF 635 (Sigma SAB4600131). Bis-benzamide (H33258, 1.2 µg/mL; Sigma-Aldrich Corp., St. Louis, MO, USA) was added to aid visualization of cell nuclei.

Dissection
After overnight immersion-fixation at 4°C, the globes were immersed in 0.1 M phosphate buffer for 30 minutes and then dissected. The ONH region was dissected free from the back of the globe with the retina where it was still intact and present. The ONH region was then dissected longitudinally or transversely with the purpose to observe the vascular distribution pattern from the arteries perfused. As far as possible, the RPE was removed from the choroid around the optic disc region using buffer moistened cotton wool buds. Thick slices of longitudinally or transversely cut ONH and optic nerve were made using a sharp razor blade. The thick slices ranged from 0.5 to 1 mm in thickness.

The retina, choroid, thick slices from optic nerve, and ONH were mounted and coverslipped on glass slides using RapiClear 1.47 or 1.52 or 1.55 (RC147001, 155001, and 152001; Sunjin Lab. Co., Hsinchu City, Taiwan) as the clearing agent. iSpacer (IS003 and IS004; Sunjin Lab. Co.) was used depending on the thickness requirement. The specimens were then imaged using a Nikon C1 confocal system (four solid state lasers emitting λ 405, 488, 561, and 637 nm) in combination with a Nikon 90i microscope (Nikon, Lidcombe, NSW, Australia).

Imaging and Processing
Image stacks were acquired using Plan Apo objective lenses at magnifications of ×2, ×4, ×10, and ×20 using the EZ-C1 confocal software. The image stacks were processed, viewed, and snapshot taken with Imaris (version 7.6.1; Bitplane, Zurich, Switzerland) and Image Pro Plus (Version 7.1; Media Cybernetics, Rockville, MD, USA) software packages.

RESULTS
Eighteen donor eyes were deemed adequate for identifying an inter-relationship of the arterial supply for the retina, choroid.
and ONH. The CRA was cannulated just before it entered the optic nerve trunk approximately 10 mm posterior to the globe. SPCAs were distinguished from long PCAs (LPCAs), and as far as possible the temporal SPCAs close to ONH were identified for cannulation. Overlap in arterial downstream supply was evidenced by concurrent appearance of dual or triple labels in downstream microvessels originating from different sources (CRA or SPCAs).

CRA-Only Perfusion

Figure 2 shows a typical pattern of microvasculature labeling from eyes perfused through the CRA only. Donor eye C was perfused via the CRA only and labeled for VE-Cadherin (VEC, red), eNOS (green), and F-Actin (blue). The fluorescent probes perfused were visualized in the CRA and CRV in the longitudinal section of the ONH as expected (Fig. 2A, a projected image of all three labels). Interestingly, in addition to the retinal microvasculature (shown by VEC and eNOS; Fig. 2B), the choroidal vasculature in both the temporal (shown by VEC; Fig. 2C) and nasal sides (shown by Actin and eNOS dual labels; Fig. 2D) were also labeled. Other vessels labeled included longitudinal branches embracing the ONH peripherally, transverse vessels in pretaminar cribrosa (PrCL) and lamina cribrosa (LC) (Fig. 2A), scleral paraoptic arterioles, choroidal vessels, and longitudinal vessel plexus within the optic nerve trunk.

SPCA-Only Perfusion

Six donor eyes were perfusion-labeled via the SPCAs only. The retinas in two of the donor eyes were destroyed in the process of vitreous retrieval and therefore not available. Figure 3 shows the results from one donor eye where a temporal SPCA was cannulated for perfusion labeling. A combination of phalloidin Alexa Fluor 635 (Pha-AF635), calponin indirectly tagged with Alexa Fluor 488 (Calponin-AF488), and VE-Cadherin (VEC) indirectly tagged using Alexa Fluor 555 (AF555) were used. To illustrate the inter-relationship of the arterial supply to the retina, choroid, and the ONH, the ONH was longitudinally and slightly obliquely sectioned (Fig. 3A). Positive labeling of choroidal arterioles was observed reflecting a segmental perfusion pattern as expected. There were abundant vessels that were positively labeled in the ONH particularly in LC and post-LC regions (Fig. 3A). All these vessels were differentially labeled by each probe (Figs. 5B–3D) in the LC region. The CRV in the center of the ONH showed positive labeling (Fig. 3A) particularly for calponin and VEC. Other than the two retinas that were destroyed, the remaining four donor retinas all
showed positive fluorescent probes in the retinal vessels perfused via the SPCAs only. These results suggest that communications between choroidal and retinal circulations exist.

Dual Cannulation of CRA and SPCAs

Nine donor eyes received dual or triple cannulation at the CRA and one or more of the SPCAs. Two of the eyes were not included in analysis due to overlap in wavelength of probes perfused via the two-arteriole sources. In the other seven eyes, probes perfused via the CRA were present in choroidal vessels. In six of these donor eyes (Table 2), labels perfused via the SPCA(s) were also present on retinal vessels, indicating communication between the two arteriole sources.

Figure 4 shows both probes were detected on retinal vessels when the probes were delivered via different arterioles; Pha-TRITC delivered via the CRA and phalloidin FITC (Pha-FITC) delivered via temporal SPCA. Classical teaching suggests that there is no direct communication between retinal and choroidal circulation. Our results suggest that it is very likely that there are communications in the ONH resulting in the Pha-TRITC probe (delivered via the CRA) being found in the choroid, as well as in the CRV.

**TABLE 2.** Results From Dual Cannulation of CRA and SPCAs of Donor Eyes F to L

<table>
<thead>
<tr>
<th>Eye</th>
<th>Retina</th>
<th>Choroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>Dual source of labels in RPCs and retinal vessels</td>
<td>Dual source of labeling observed in choroid</td>
</tr>
<tr>
<td>G</td>
<td>Dual source of labels in RPCs and retinal vessels</td>
<td>Dual source of labeling observed in choroid</td>
</tr>
<tr>
<td>H</td>
<td>Dual source of labels in RPCs and retinal vessels</td>
<td>Dual source of labeling observed in choroid</td>
</tr>
<tr>
<td>I</td>
<td>Dual source of labels in RPCs and retinal vessels</td>
<td>Dual source of labeling observed in choroid</td>
</tr>
<tr>
<td>J</td>
<td>Triple labels in RPCs and retinal vessels</td>
<td>Triple source of labels in choroid</td>
</tr>
<tr>
<td>K</td>
<td>Single source of label observed in RPCs and vessels</td>
<td>Triple source of labels in choroid</td>
</tr>
<tr>
<td>L</td>
<td>Dual source of labels in RPCs and retinal vessels</td>
<td>Dual source of labeling observed in choroid</td>
</tr>
</tbody>
</table>

Results from donor eyes D and E are omitted due to overlap in probe signals. RPC, radial peripapillary capillaries.
choroidal vasculature and the Pha-FITC probe (delivered via the SPCA) being found in the retinal vasculature. To identify the possible communications, we dissected the ONH either longitudinally, obliquely, or transversely after dual perfusion from CRA and SPCA. Figure 5 shows an ONH that was obliquely-transversely dissected. Similar to the results showed in Figure 4, the retinal vasculature was labeled by both lectin-TRITC via CRA and lectin-FITC via SPCA. Interestingly, numerous choroidal vasculature and the Pha-FITC probe (delivered via the SPCA) being found in the retinal vasculature. To identify the possible communications, we dissected the ONH either longitudinally, obliquely, or transversely after dual perfusion...
microvessels and capillaries in LC region of the ONH were labeled with both probes, indicating that communications between CRA and SPCA exist in this region. Segmental labeling appeared in the choroidal vasculature with more extensive labeling of FITC (SPCA perfused) compared with TRITC (CRA perfused). The images in Figure 6 were taken from both thick longitudinal and transverse slices of the optic nerve and ONH regions after fluorescent probes were delivered via the CRA (lectin-TRITC) and tSPCA (lectin-FITC). Lectin-TRITC labeled CRA and some microvessels and capillaries in the optic nerve trunk, whereas only a few FITC-labeled microvessels were found in the peripheral optic nerve trunk. Larger and dense blood vessels can be seen in the pre-LC and LC. Both retinal and choroidal vessels (Figs. 6G, 6H) were dual labeled in this eye. To further confirm our findings, we studied the vascular labeling distribution in the ONH after the probes were delivered through the CRA and two SPCAs (Fig. 7). Three different fluorescent probes were delivered via the CRA (lectin-TRITC), a tSPCA (lectin-FITC), and a second SPCA (phalloidin AF647 for F-Actin).

Single channel images of the three probes (Figs. 7A–7C) in the longitudinal section of the ONH showed labeling of feeding vessels within sclera, the CRA, LC region, choroidal vessels, and retinal microvasculature attached to the optic disc region. The images of the three probes in the post-LC vessels (Figs. 7D–7F) show all three labels reached vessels in the paraoptic scleral region. Lectin-TRITC delivered via the CRA may be seen in microvessels within the vicinity of the CRA, as well as large vessels extending from the optic nerve. These larger vessels form part of the circle of Zinn-Haller and were labeled positively by lectin-FITC and Pha-AF647 delivered via the two SPCAs. In the images of the three probes in the LC region (Figs. 7G–7I), some larger radiating vessels were labeled, with all three probes indicating cross-communication. In the lectin-TRITC probe delivered via the CRA (Fig. 7H), positive labeling in several radially transverse vessels connected to the larger vessels in the paraoptic sclera. The large scleral vessels were also positively labeled by lectin-FITC probe delivered via one of the SPCAs, showing more extensive cross-communication.

In addition to the general findings described above, an unusual finding was noted in some eyes (Fig. 8). Specifically, branches of retinal artery traveling back toward the ONH to supply the ONH in three donor eyes were unexpected. All three ONHs in Figure 8 showed a branch from the major retinal artery traveling backward to supply the optic disc.

**DISCUSSION**

The major finding in this study is the demonstration of a close inter-relationship in the blood supply to the ONH, retina, and choroid of the human eye. The presence of such a close inter-relationship suggests that the blood flow in the ONH, choroid, and/or retina can be affected by changes in an individual arterial pathway in both normal and pathologic conditions.

Numerous studies on the anatomy (casting), histology (serial sectioning), and fluorescence angiography of human and monkey ocular circulations have been performed, but controversies remain in the supply origin of ocular microvasculature. Traditionally, the retinal and choroidal circulations are considered end-arterial systems without anastomoses, with the CRA supplying the retinal circulation and the SPCAs supplying the choroidal circulation. Both the CRA and SPCAs derived from branching of the intraorbital ophthalmic artery, and both are known to give off optic nerve arterioles.
branches providing the blood supply of the ONH. However, Hayreh has emphasized that there are marked interindividual variations in the blood supply of the ONH. These variations included the anatomical pattern of blood supply, the pattern of PCA circulation, and the blood flow distribution. This study did not attempt to identify these anatomic and functional variations. We focused instead on determining whether there is a close inter-relationship of blood supply to the retina,

**FIGURE 7.** Distribution of probes delivered through CRA and two SPCAs. Donor eye J had three different fluorescent probes delivered via the CRA (lectin-TRITC), a SPCA (lectin-FITC) and a second SPCA (phalloidin AF647 for F-Actin). (A–C) Low-magnification, single channel images of the three probes in the longitudinal section of the ONH. Phalloidin-AF647 may be seen labeling feeding vessels within sclera, the CRA, LC region, choroidal vessels, and retinal microvasculature attached to the optic disc region. Lectin-TRITC labeled the CRA, CRV, retinal microvasculature, and choroidal vasculature at the optic disc region. Lectin-FITC labeled feeding vessels into the choroid, choroidal vasculature, and transverse vessels within the LC region, as well as longitudinal and transverse vessels in the optic nerve. (D–F) Images of the three probes in post-LC vessels. All three labels were observed in vessels in the paraoptic scleral region. Lectin-TRITC delivered via the CRA may be seen in microvessels within the vicinity of CRA, as well as large vessels (arrow) extending from the optic nerve. These larger vessels form part of the circle of Zinn-Haller and are labeled positively by lectin-FITC and Pha-AF647 delivered via the two SPCAs. (G–I) Images of the three probes in the LC region. Arrow points to one of the larger radiating vessels that have been labeled with all three probes, indicating cross-communication. (H) Lectin-TRITC probe delivered via the CRA showing positive labeling in several radially transverse vessels connected to the larger vessels in the para-optic sclera. The large scleral vessels are also positively labeled by lectin-FITC probe delivered via one of the SPCA (I), showing more extensive cross-communication. Scale bars denote 400 μm for all images.
choroid, and ONH using microcannulation, perfusion, and labeling techniques used previously by our group.\textsuperscript{19,20,23–26} Our results clearly demonstrate fluorescent probes perfused via the CRA appear in some choroidal vessels, indicating that blood flow via the CRA can travel to both the retinal and the choroidal vasculature. Conversely, probes perfused via SPCAs also appear in retinal vessels, indicating that blood flow via the SPCAs can supply the vasculature in the retina, choroid, and the ONH.

We considered the possible contribution by cilioretinal arteries, which are known to supply anywhere from 25\% to 100\%\textsuperscript{27–29} of the retinal microvasculature when present. Most of the reports to date indicated the SPCAs to be the origin of the cilioretinal arteries. Although we did not trace the pathway of the SPCA perfusion, given the high occurrence of cilioretinal arteries in the population, it is possible for dye to have reached the retinal vasculature via a cilioretinal artery branch from the perfused SPCA. Results from dual cannulation of CRA and selected SPCAs showed probes from both origins appeared in both retinal and choroidal vessels. Dual labeling of retinal and choroidal vessels was observed in numerous vessels in the pre-LC and LC regions of the ONH, indicating extensive potential collateral pathways. These dual-labeled vessels may or may not act like the watershed zone described by Hayreh. A watershed zone is defined as the border between the distribution territories between two end arteries,\textsuperscript{18} such as the CRA and SPCAs.

Arguably, the existence of an interplay between the blood supply to the ONH, retina, and choroid adds to the complexity of intraocular blood distribution control mechanisms. Vascular networks, especially terminal vascular beds of the microcirculation, may no longer be simplistically portrayed as symmetrical arrays of vessel segments in which all flow pathways are equivalent. Real vascular networks have a large degree of topologic and structural heterogeneity.\textsuperscript{30} The challenge to understand how efficient blood flow distribution is achieved for high metabolic demand tissues in the retina and the ONH during physiologic conditions remains a fundamental biological question. As feeding vessels, the CRA in the post-LC region and the SPCAs receive input from the autonomic nervous system\textsuperscript{31,32} and probably play an important role in maintaining the stability of perfusion in the ocular region in response to systemic changes. It has also been reported that there is a lack of the ONH vasculature innervation anterior to LC.\textsuperscript{32,33} Therefore, autoregulation mechanisms including metabolic, myogenic, humoral, and endothelial mediators are likely the main players in the local regulation of the ONH blood supply. The factors in play to maintain homeostasis in the optic nerve region are therefore manifold and complicated. Insight into the extent and pattern of anastomosis between the CRA and SPCAs within the ONH may provide us with more understanding of the delicate controls in this region. Indeed, knowledge of the inter-relationship of the blood supply to the retina, choroid, and ONH could be highly relevant to the pathogenesis of the ONH, retinal, and choroidal ischemic diseases.

The presence of extensive anastomoses between the two supply sources as demonstrated in this study means that in the event of occlusion, there is potential overlapping support available. Perhaps the extensive connections observed, especially in the LC and prelaminar regions, may be analogous to the umbra/penumbra observed in cerebrovascular occlusion patients in which the region of ischemia can be minimized by
treatments to encourage flow from surrounding vessels. Further advancements in imaging and blood flow detection techniques will help to illuminate these unknowns. This knowledge adds to our understanding of the vessel distribution and supply relationship in the human ONH and retina.

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**References**


