Optic Nerve and Peripapillary Choroidal Microvasculature of the Rat Eye

Kazuhisa Sugiyama, Zbao-Bin Gu, Chizuru Kawase, Tetsuya Yamamoto, and Yoshiaki Kitazawa

PURPOSE. To investigate the three-dimensional microvascular anatomy of the optic nerve and peripapillary choroid in the rat eye.

METHODS. Gross vascular anatomy of the posterior eye segment of Wistar rats was studied in serial microsections with a light microscope. The optic nerve and peripapillary choroidal vessels were sequentially microdissected, using methylmethacrylate corrosion microvascular castings, and were examined with a scanning electron microscope to determine the three-dimensional relationships of the vessels.

RESULTS. The posterior ciliary artery traveled along the inferior side of the optic nerve sheath, directly entered the optic nerve head, and divided into three branches: the central retinal artery and medial and lateral long posterior ciliary arteries, which provided several short branches to the choroid. The optic nerve head vasculature was consistently nourished by a recurrent arteriole from the central retinal artery and an arteriole from the choroidal artery at the peripapillary choroid. The central retinal vein flowed into a venous anastomosis along the optic disc border of the peripapillary choroid. Capillaries within the optic nerve drained into the central retinal vein, the marginal venous anastomosis of the peripapillary choroid, and the pial veins, all of which flowed into the posterior ciliary veins along the optic nerve sheath.

CONCLUSIONS. The findings illustrate vascular anatomic differences in optic nerve and peripapillary choroidal microcirculation between rat and human. In rats, the peripapillary choroid plays a significant role in both blood supply and venous drainage of the optic nerve head. The central retinal artery also contributes to the optic nerve head circulation. (Invest Ophthalmol Vis Sci. 1999; 40:3084–3090)

The microvascular supply and drainage of the optic nerve and peripapillary choroid have been studied in rabbit, nonhuman primate, and human by physiologically controlled microvascular corrosion castings, which permanently replicate the anatomic condition of vascular beds under the physiologic conditions at the time of plastic injection. Such a plastic model of the ocular vasculature appears to preserve the vascular tone and has been used to demonstrate statistically significant differences in vascular calibers between eyes treated with adrenergic drugs and contralateral control eyes in rabbits. Our previous work has shown that the main arterial blood supply to the anterior optic nerve is from its periphery (branches of short posterior ciliary arteries) and that the central retinal vein provides the sole venous drainage route of optic nerve circulation in nonhuman primates and human. Our past results suggest that the direction of the optic nerve blood flow is from the peripheral blood supply toward the central venous drainage.

Recently, detailed methods to increase intraocular pressure in laboratory rats have been published, and rats have been extensively used as a readily available animal model of glaucoma and as an optic nerve axotomy or crush model for neuroprotection studies with relevance to glaucoma. However, ocular hypertension or optic nerve injury may cause ischemia or disturbance of the microcirculation in the optic nerve head, retina, or choroid in addition to direct nerve damage. Detailing the three-dimensional microvascular anatomy of normal laboratory rats is crucial to establishing rat models for glaucoma or optic nerve injury. Researchers should also know the vascular anatomic differences between rat and human before extrapolating results from rat models to human conditions. We first examined serial microsections of rat optic nerve by light microscopy to determine the anatomic position of various blood vessels with respect to the surrounding structures because surrounding tissue had been removed from the vascular castings. Then, we investigated the three-dimensional microvascular anatomy of the optic nerve and peripapillary choroid using scanning electron microscopy of microvascular castings from laboratory rats. The results provide a basis for understanding the microcirculation of the rat optic nerve head region and the vascular anatomic differences between rat and human eyes.

MATERIALS AND METHODS

All our experimental procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.
search. We used 45 Wistar rats 9 weeks after birth, each weighing 250 to 300 g, which had no signs of ocular inflammation by slit lamp examination.

Enucleated eyes (10 eyes of five rats) were placed in a mixture of freshly prepared 2% paraformaldehyde, 2% glutaraldehyde, and 0.1 M phosphate buffer (pH 7.2). After 5 minutes, a slit was made 1 to 2 mm posterior to the limbus with a number 11 scalpel blade, and the eyes were returned to the fixative. The eyes were processed through graded solutions of ethanol and xylene and then embedded in paraffin. For histologic study, 3-μm serial cross-sections (five eyes) and longitudinal sections (five eyes) of the optic nerve were obtained and stained with hematoxylin and eosin. These serial sections were observed through a photomicroscope (Axioskop, Carl Zeiss, Oberkochen, Germany).

We modified luminal microvascular corrosion casting techniques previously described for use in rabbits and rats and examined the microvasculature of the optic nerve and posterior segment of the eyes in 40 rats. In brief, the castings of the ocular vasculature were obtained under controlled physiologic conditions. Rats were anesthetized by intraperitoneal injection of ketamine (75 mg/kg body weight) and xylazine (7.5 mg/kg body weight). Methacrylate injection media (Batson’s 17; Polyscience, Warrington, PA) was modified to reduce the viscosity to 11 centipoise (cp), a level only slightly higher than that of heparinized blood (8 cp). To avoid ischemia, the respiratory system was preserved until the moment of the plastic injection. The plastic was injected into the superior circulation through the ascending aorta from a cannula inserted into the abdominal aorta. Immediately before starting the injection, we opened the thorax and cut the right atrium as a drainage route of the superior circulation. The plastic was injected at physiologic temperature (37°C), with a slightly higher perfusion pressure (150–180 mm Hg) than physiologic blood pressure. The pressure was monitored with a transducer (AP-641G; Nihon Kohden, Tokyo, Japan). The injection pressure was maintained for 5 to 10 minutes, until the plastic began to polymerize. Two hours after injection, the eyes were enucleated, stored overnight in 10% buffered formalin to complete the polymerization, and corroded in 6 M potassium hydroxide at 50°C for 2 or 3 days. The plastic vascular castings thus created were carefully rinsed with a mild flow of distilled water using a pipette and then dehydrated in graded solutions of ethanol. Finally, the ocular castings were desiccated by t-butyl alcohol freeze-drying. Whole globe vascular castings were hemisectioned at the equator, and the posterior segments were mounted on stubs, sputter coated with gold-palladium (JFC-1500, ION sputtering device; JEOL, Tokyo, Japan), and examined with a scanning electron microscope (JSM-5410LV; JEOL).

Despite the controlled conditions that consistently produce uniform filling of the ciliary body and choroidal vasculature, complete methacrylate filling is more variable in the optic nerve and retinal vasculature. Because of these difficulties, we examined only the castings (26 eyes of 20 rats) in which the retinurnal network of the optic nerve was completely filled.

The optic nerve castings were viewed from both the anterior and posterior aspects of the globe. With an insect-dissection needle and microscissors, the optic nerve and peri-papillary choroidal vessels were sequentially removed to determine specific three-dimensional relationships of the vessels. This technique was previously described using ocular castings of rabbit and nonhuman primate. After removal of the superficial layers, the castings were recoated with gold-palladium to obtain electron microscopic scans of deeper, previously inaccessible layers.

**RESULTS**

**Gross Vascular Anatomy**

The cross-sectional histology of the optic nerve and posterior view of the ocular castings revealed that one artery existed in the thick dura of the optic nerve and traveled along the inferior side of optic nerve without giving off any branches (Figs. 1, 2). This artery directly entered the inferior side of the optic nerve head and provided two long posterior ciliary arteries in the sclera along the horizontal meridian on the medial and lateral sides of the choroid (Figs. 2, 3). The choroid of the posterior segment, including peripapillary choroid, was nourished by several short branches from long posterior ciliary arteries (Fig. 2). Careful microdissection revealed that the artery finally became a central retinal artery after branching into the two long posterior ciliary arteries (Figs. 3, 4). This artery supplied the entire uveal vasculature including the retinal vasculature; therefore, this artery should be called the posterior ciliary artery.

**Optic Nerve Head and Peripapillary Choroidal Microvasculature**

The surrounding tissue had been removed from the casting; therefore, histologic methods were used to determine the
anatomic relationship of the various blood vessels to surrounding structures. Figure 5 depicts the longitudinal section of the rat anterior optic nerve, revealing a bottleneck configuration.

The rat optic nerve head has laminar beams (Fig. 3) identical with the primate lamina cribrosa as described in a previous study. In the present study, we divided the rat anterior optic nerve into four anatomic regions in accordance with the recognized regions of the primate optic nerve head. The most anterior zone of the optic nerve is the superficial nerve fiber layer region. Immediately posterior to this is the prelaminar region, which lies adjacent to the peripapillary choroid. More posteriorly, the laminar region is continuous with the sclera and is composed of lamina cribrosa. Finally, the retrolaminar region lies posterior to the lamina cribrosa.

The anterior view of the posterior segment of the ocular casting showed that the central retinal artery supplied the retinal vasculature and the surface nerve fiber layer of the optic nerve head (Fig. 6). The lateral view of the posterior segment of the ocular casting showed the optic nerve head and peri-
papillary choroid (Fig. 7A). We carefully microdissected part of the choroidal vasculature to observe the prelaminar and laminar regions of the optic nerve head (Fig. 7B). One branch of the central retinal artery consistently provided a recurrent arteriole to the prelaminar, laminar, and retrolaminar regions of the optic nerve head. The capillary network within the optic nerve was continuous from the retrolaminar region to the retinal vasculature.

The posterior view of the ocular casting and cross-sectional histology of the laminar region of the optic nerve head demonstrated that in addition to the vortex system, another independent venous system of the posterior choroid formed an incomplete venous circle around the optic nerve head in the sclera (Figs. 8A, 8B). This venous circle drained into the posterior ciliary veins parallel to the posterior ciliary artery in the inferior side of optic nerve dura (Fig. 8A; see also Figs. 1, 2). After removing some choroidal vasculature, the venous circle, and optic nerve capillaries, we were able to observe that the central retinal vein emptied into a marginal venous anastomosis along the optic disc border of the peripapillary choroid (Fig. 4). The marginal venous anastomosis finally flowed into the posterior ciliary veins. Capillaries in the retinal vasculature, surface nerve fiber layer, and prelaminar region of the optic nerve head drained into the central retinal vein (Fig. 7B). Capillaries within the prelaminar and laminar regions of the optic nerve head drained into the marginal venous anastomosis of the peripapillary choroid (Fig. 7A). In the laminar and retrolaminar regions, most of the venous drainage was to pial veins, which flowed into the posterior ciliary veins (Fig. 9).

**DISCUSSION**

Although rat posterior choroidal vasculature and posterior ciliary vein have been described, this is the first overall in-depth study of the optic nerve head and peripapillary choroidal microvasculature of the rat eye. Because the methylmethacrylate filling of the rat optic nerve vessels is more variable than the filling of the optic nerve vessels in larger mammals, making a complete microvascular casting of the optic nerve in the rat

---

**FIGURE 6.** The anterior view of the casting showing the arterial supply (arrows) of the surface nerve fiber layer of the optic disc. CRA, central retinal artery; CRV, central retinal vein. Bar, 100 μm.

**FIGURE 7.** (A) From the lateral view of the casting, the arrows indicate that the capillary network of the optic nerve head flows into the marginal venous anastomosis (MVA). (B) Part of the peripapillary choroidal vasculature was removed. The small arrows indicate the branch of the central retinal artery (CRA). The large arrows denote the branch from the choroidal artery (CA). The double arrows show that capillaries of the prelaminar region drain into the central retinal vein (CRV). ON, optic nerve; CH, choroid; CA, choroidal artery; Re, retinal vasculature. Bar, 100 μm.
eye is very difficult. The vascular castings of a rat optic nerve are much smaller than those from rabbit, nonhuman primate, or human, making them weak and fragile. Even when complete microvascular filling of rat optic nerve and retinal vasculature is obtained using previously described methods, rinsing with water and air drying could destroy detail at the capillary level of complicated vascular castings, although such destruction has not occurred in larger ocular castings. In the present study, we carefully rinsed the ocular castings with a mild flow of distilled water, by means of a pipette. The castings were then dehydrated in graded solutions of ethanol and desiccated by tbutyl alcohol freeze-drying. These procedures enabled us to preserve fragile castings. Additionally, it is extremely important to reduce the viscosity of the casting media to replicate the complete optic nerve microvasculature without insufficient filling or extravasation of media. A modified methylmethacrylate with a viscosity of 11 cp was used. This plastic medium was only slightly more viscous than heparinized venous blood (8 cp) at the time of injection. Maintaining a physiologic temperature of the injection media and keeping the respiratory system functioning until the moment of plastic injection were also crucial to preserving vascular tone.

The precise anatomic relationships of the vessels that perfuse and drain the optic nerve and peripapillary choroidal regions are difficult to study because of their inaccessibility and the complexity of their angioarchitecture. When methylmethacrylate luminal corrosion castings are viewed with a scanning electron microscope, only surface vessels are clearly visible. However, the use of a sequential microdissection technique allowed detailed inspection of the inner three-dimensional angioarchitecture of the anterior optic nerve and peripapillary choroid.

Rat posterior ocular vasculature has a unique and simple angioarchitecture compared with that of rabbit or primates. In rabbit or primates, medial and lateral posterior ciliary arteries are present apart from the optic nerve; however, the present study shows that in rats, the posterior ciliary artery travels in the inferior side of the optic nerve sheath toward the optic nerve head. The posterior ciliary artery is derived from the inferior branch of the ophthalmic artery in the rat eye. This study also confirmed that the posterior ciliary artery gives off two long posterior ciliary arteries and a central retinal artery at

FIGURE 8. (A) Posterior view of the casting, with arrows indicating the incomplete venous circle, which drains into the posterior ciliary vein (PCV). (B) Cross-section of optic nerve head in the scleral level showing the incomplete venous circle around the optic nerve head (arrows). CH, choroid; CRA, central retinal artery, CRV, central retinal vein; PCA, posterior ciliary artery; LPCA, long posterior ciliary artery; CA, choroidal artery; ON, optic nerve; LC, lamina cribrosa; Sc, sclera. Bar, (A) 500 μm; (B) 100 μm.

FIGURE 9. The lateral view of the retrolaminar region of the optic nerve casting demonstrates that capillaries of the optic nerve flow into the pial venous network (arrows), which drains into the posterior ciliary vein (PCV). PCA, posterior ciliary artery. Bar, 100 μm.
the optic nerve head region, as previously described.24 The two long posterior ciliary arteries provide several branches to the choroidal vasculature and finally supply the iris and ciliary body vasculature.20,27,28 Unlike in the primate eye, in the rat eye two long posterior ciliary arteries supply the entire uveal vasculature, and there is no short posterior ciliary artery. These anatomic findings suggest that the rat posterior ciliary artery is a terminal artery to the eyeball and that optic nerve axotomy inevitably cuts the posterior ciliary artery and produces ischemia in the overall ocular vasculature. Crushing of the optic nerve, depending on the force involved, must cause occlusion or stenosis of the posterior ciliary artery. Clipping of the optic nerve is dependent on instant and limited force to result in mechanical damage to the axons without prolonged ischemia to the entire ocular vasculature. In the primate eye, axotomy or crushing at proper sites on the anterior optic nerve may not cause ischemia in retinal or choroidal vasculature, because the posterior ciliary arteries are separated from the anterior optic nerve, and the central retinal artery and vein enter the optic nerve 3 to 5 mm behind the globe.2,3,29–33

The present study clearly demonstrates that the central retinal artery in the rat eye contributes to the optic nerve blood supply, not only in the surface nerve fiber layer but also in the prelaminar, laminar, and retrolaminar regions. In contrast, the central retinal artery in human and nonhuman primates primarily supplies blood to the surface nerve fiber layer with a limited contribution to the retrolaminar region.2,3,29 Moreover, in the human eye, the pial and centripetal branches from circle of Zinn-Haller (derived from short posterior ciliary arteries) and direct branches from posterior ciliary arteries are the principal supply to the prelaminar, laminar, and retrolaminar regions.3,30–33 Our results showed that the circle of Zinn-Haller was absent in the rat eye and an arteriolar branch from a choroidal artery at the peripapillary choroid supplied the prelaminar, laminar, and retrolaminar regions along with an arteriolar branch from the central retinal artery. Only two arteriolar perfused the rat optic nerve head capillaries, suggesting that the rat optic nerve head may be more vulnerable to ischemia than that of the human.

The present study elucidates that the venous drainage of the rat optic nerve head is through a marginal venous anastomosis of the peripapillary choroid in the prelaminar and lamina regions and through pial veins in the laminar and retrolaminar regions. The central retinal vein collected venous tributaries from the retina, surface nerve fiber layer, and prelaminar regions of the optic nerve head, then drained into the marginal venous anastomosis of the peripapillary choroid and the posterior ciliary veins. These anatomic findings demonstrate that the marginal venous anastomosis of the peripapillary choroid plays a significant role in the venous drainage of the optic nerve head and the retinal vasculature. The present study and a previous report indicate that posterior ciliary veins as well as vortex veins provide venous drainage of the posterior choroid in the rat.24

Finally, this study shows that the central retinal artery and the choroidal artery of the peripapillary choroid present a double arterial vascular supply to the rat optic nerve head. The venous outflow of the optic nerve head is by means of the marginal venous anastomosis of the peripapillary choroid and pial veins. Thus, the peripapillary choroid plays a key role in the rat optic nerve head microcirculation. The understanding of the vascular anatomic differences between rat and human eyes enables the proper interpretation of results from rat experimental models for extrapolation to humans.

Acknowledgments

The authors thank the Biological Department, R & D Division, Menicon Co., Ltd., Japan for providing scanning electron microscopic facilities and Ai Nishizawa for her technical assistance.

References


