The homogeneous structure of blood vessels in the vascular tree of *Macaca mulatta* iris

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This study reports the results of a systematic analysis of the iris vasculature in *Macaca mulatta*. Through the application of a variety of morphologic procedures it has been demonstrated that in rhesus monkeys, all iridal vessels, regardless of their diameter, have a similar structure. Their walls consist of (1) endothelial cells in a continuous layer resting on a basal lamina, which are provided with a small number of blunt luminal protrusions and slender basal lamellae; (2) pericytes sandwiched between two layers of the basal lamina, which are characterized by a smooth basal surface and adluminal processes that interdigitate with the basal leaflets of the endothelial cells; and (3) an adventitia of fibroblasts, melanocytes, and occasional macrophages arranged in one or more layers. Typical smooth muscle cells are not found in any vessels of *M. mulatta* iris. Thus the vessels of the rhesus monkey iris have a remarkably homogeneous morphologic appearance and cannot be classified according to the traditional criteria for arterioles, capillaries, and venules. (INVEST OPHTHALMOL VIS SCI 22:279-291, 1982.)

Key words: blood vessels, iris, monkey, electron microscopy

Knowledge of the vascular pattern of the iris and the structure of individual iridal vessels is an important prerequisite to the understanding of their function in both normal and pathologic conditions. Furthermore, such information is essential for the proper interpretation of fluorescein angiographic studies. Although some information exists on the ultrastructure and permeability properties of small iris vessels in different monkey species, the vascular system of the monkey iris has never been systematically analyzed. The goals of this study were to determine the vascular pattern of the iris in *Macaca mulatta* and to ultrastructurally characterize the individual vessels.
Our results demonstrate that the vascular network of the rhesus monkey iris is different from that classically described in man.3

Materials and methods

Animals. A total of nine rhesus monkeys (M. mulatta) of both sexes were used. These animals were from 6 months to 15 years old.

All animals were sedated with an intramuscular injection of ketamine HCL (10 mg/kg) and maintained in deep anesthesia with sodium pentobarbital (30 mg/kg). One young and one adult monkey were subsequently perfused via the left ventricle of the heart with diluted fixative fluid4 and the eyes were enucleated. In the remaining animals, horseradish peroxidase (Type II; Sigma, 500 mg/kg body weight dissolved in 3 to 5 ml of phosphate-buffered saline, pH 7.2) was slowly injected into the small saphenous vein. After 10 to 15 min the animals were sacrificed with an overdose of anesthetic and the eyes were enucleated. Most eyes were cut at the equator and immersed in fixative of the heart with diluted fixative fluid4 and the eyes were enucleated. Most eyes were cut at the equator and immersed in fixative fluid (2% paraformaldehyde and 2.5% glutaraldehyde in 0.1M cacodylate buffer, pH 7.3). The eyes of one animal were opened and fixed with 2% OsO4 in Millonig's phosphate buffer, pH 7.3, containing 0.4% CaCl2.

Whole-mount preparations of the iris vasculature. To demonstrate the vascular tree in the heavily pigmented irides of these animals, we utilized our previously reported staining technique.5

Thin sections of isolated large iris vessels. After fixation and washing, some specimens were sectioned (200 μm) with a Smith-Farquhar tissue chopper and were reacted to demonstrate peroxidase activity as previously described.2 In these sections the largest iris vessels were recognized and individually dissected from the iris stroma. The isolated vessels were postfixed in 1% OsO4 and 1.5% potassium ferrocyanide in distilled water, dehydrated, and embedded in an Epon-Araldite mixture. Thin sections were cut in both the longitudinal and transverse planes of the vessels.

Preparation of consecutive thick and thin sections of iris vessels. From other chopper sections, not reacted for demonstration of peroxidase activity, sequential light microscopic (1 to 2 μm sections) and thin sections for electron microscopic examination were made for correlated study. These sections were used to compare the structure of iris vessels of different diameters and to determine whether differing positions within the iris influenced the structure of their walls.

Serial sections of iris wedges from the pupillary margin to the major circle of the iris. Remaining uveal specimens were cut into a series of wedges. From these wedges serial light microscopic sections were cut from the pupillary margin to the pars plana. In this manner individual vessels could be followed to their origin, changes in the structure of their walls could be observed, and the major circle of the iris could be positively identified and followed.

Staining and microscopy. Light microscopic sections were stained with toluidine blue and thin sections were stained with uranyl acetate and lead citrate. Thin-sectioned material was examined with either an RCA-3G or AEI-6B electron microscope.

Scanning electron microscopy. For scanning electron microscopy, wedges of iris were prepared by the method of Malick and Wilson6 (OTOTO method) and examined in an ISI-60 scanning electron microscope operated at 15 kV.

Results

Iris whole-mount preparations. Microscopic examination of whole-mount preparations clearly demonstrated the general vascular pattern of the iris (Fig. 1). A series of large radial vessels was seen; many of them followed a tortuous path when the iris was moderately mydriatic. The largest vessels were either formed by, or gave rise to, intermediate vessels in the iris stroma; branching often began immediately at the root of the iris. Alternatively, the large vessels continued directly to the pupillary margin, with only minimal decrement in diameter, and arborized into a continuous arcade of small vessels. The large radial vessels did not give rise, however, to a continuous anastomotic circle in the collarette region. In fact, there was no greater number of vessels oriented in a circumferential fashion in the collarette region than that elsewhere in the iris, thus indicating that in this species, a minor circle of the iris is absent. At the collarette, however, there was a tendency for vessels of intermediate caliber to emerge from the underlying stroma to appear at the anterior iris surface and thus mimic the appearance of the discontinuous minor circle classically described in
Fig. 1. Whole-mount preparation of *M. mulatta* iris in moderate mydriasis. A series of large radial vessels gives rise to a complex network of intermediate and small vessels throughout the length of the iris. A continuous arcade of small vessels is seen at the pupillary margin. Note the absence of a minor circle of the iris in the collarette region (asterisks). (×58.)
the human iris (Fig. 2). Such vessels likely contributed to the vascular supply of the pupillary membrane, which joined the iris at the collarette during fetal development. These superficial vessels did not, however, represent either an arteriovenous anastomosis or a point of termination for large iris vessels.

Although much valuable information was derived from the whole-mount preparations, it was impossible to distinguish between arteriolar (afferent) and venular (efferent) vessels. Furthermore, preliminary examination of thin sectioned material had suggested that standard criteria used for the classification of microvessels were unsuitable for categorizing iris vessels. For these reasons iris vessels were arbitrarily divided into three groups based on their diameter. Vessels with diameters greater than 20 \( \mu m \) were classified as large. Those classified as medium were from 14 to 20 \( \mu m \), and small vessels had diameters of less than 14 \( \mu m \).

**Isolated large iris vessels.** The largest iris vessels, which had been individually dissected, had a surprisingly simple wall. In longitudinal and transverse sections the vessel wall consisted of a continuous endothelium surrounded by a continuous basal lamina, a discontinuous layer of pericytes, and an adventitia composed primarily of fibroblasts, which commonly gave rise to multiple thin lamellar processes (Fig. 3). No smooth muscle cells or internal elastic lamina were present in the walls of these vessels.

**Examination of consecutive thick and thin sections of iris vessels.** By cutting light microscopic sections (1 to 2 \( \mu m \)) and then, sequentially, thin sections for electron microscopic examination, walls of iris vessels of different diameters but of known position in the iris were readily compared. In each instance no difference was found in the structure of the walls between large, medium, and small caliber vessels (Figs. 3 to 5). The endothelium of all iris vessels was continuous, and endothelial cells possessed blunt luminal protrusions and slender basal leaflets or digitations. In all iris vessels the intercellular clefts were closed to the passage of HRP by the presence of zonulae occludentes (Fig. 3). The cytoplasmic organelles of the endothelial cells were unremarkable except for the occasional presence of rod-shaped bodies and crystalloid inclusions associated with the rough endoplasmic reticulum. These organelles were identical to those previously reported in a variety of adult and developing ocular vessels in *M. mulatta*.

The pericytes of iris vessels were similar to those found elsewhere, including retina, brain, connective tissue, and skeletal muscle. The ultrastructural characteristics of these cells were identical to those described by previous authors and included a variable complement of filaments, primarily aggregated in the cytoplasm adjacent to the adluminal plasmalemma. Furthermore, as has been reported for pericytes of small vessels in the retina, corneal limbus, and myocardium, the distribution of plasmalemmal vesicles was asymmetric; they were commonly observed at the abluminal surface of these cells but only very seldom at the adluminal surface. All pericytes observed were positioned within the basal lamina, and lamellar processes of these cells often pierced the basal lamina to closely appose or interdigitate with the basal leaflets of the endothelial cells. The adventitia of iris vessels was composed of various cellular elements surrounded by a collagen sheath. Most adventitial cells were fibroblasts and melanocytes; rare macrophages could be recognized in sections of specimens treated with diaminobenzidine and hydrogen peroxide by the endogenous peroxidase activity of lysosomes and residual bodies in their cytoplasm.

When vessels were positioned in more densely cellular stromal regions, they were invested with a thicker, more densely cellular adventitia. For instance, vessels lying near the cellular anterior border layer of the iris exhibited a thick, cellular adventitia that occasionally included nerve cell processes (Fig. 6). The structure of the adventitia was not related to vessel caliber, however, since vessels of equivalent size and situated in less densely cellular stromal regions had a correspondingly simpler adventitia (Fig. 7). The collagen sheath surrounding iris vessels in
Fig. 2. Scanning electron micrograph of *M. mulatta* iris in moderate miosis. An intermediate caliber vessel emerges from the iris stroma to branch at the anterior iris surface (arrow). The pupillary margin is seen at the bottom of the figure. (×130.)
Fig. 3. Longitudinal section of isolated radial vessel from M. mulatta iris. Horseradish peroxidase reaction product fills the lumen of the vessel, but passage of tracer into the stroma is blocked by the intercellular junctions between endothelial cells (arrow). In the vessel lumen a red blood cell with endogenous peroxidase activity is seen. The vessel has a simple wall that does not contain smooth muscle cells. The adventitia of the vessel is composed primarily of fibroblasts, which give rise to multiple thin laminar processes (arrowheads). End, Endothelium. (×8040.)
Fig. 4. Vessel from M. mulatta iris. The vessel wall is composed of a continuous endothelium (End) and basal lamina; a pericyte is seen completely surrounded by the basal lamina. The adventitia is composed of fibroblasts. (X6992.)

the rhesus monkey had an ultrastructure similar to that previously described.25–29 This sheath was composed of circularly arranged collagen fibrils, 60 to 80 nm in diameter, and was separated from the cellular portions of the adventitia by a variable space in which a sparse network of collagen fibrils, 20 to 30 nm in diameter, was seen.

Structure of the major circle of the iris. With serial sections of the iris and ciliary body the major circle of the iris could be readily identified and followed. In M. mulatta this vessel was not continuous. In thin sections the vessel wall was seen to be composed of a continuous endothelium surrounded by a continuous basal lamina and a single layer of smooth muscle cells. The adventitia of the vessel was mostly represented by fibroblasts. The average diameter of the vessel was 50 μm.

Discussion

Major circle of the iris. The vascular system of the iris in different species is quite variable. In man the major circle of the iris is traditionally described as lying wholly within the anterior portion of the ciliary body, representing a continuous arterial circle formed by the anastomoses of the anterior and long posterior ciliary arteries.3, 30, 31 In the rabbit eye the major arterial circle lies within the mid-periphery of the iris and receives no contribution from the anterior ciliary arteries.32, 33 A similar pattern is seen in guinea pigs,32 rats,35–36 and dogs.37 In man a series of radial arterioles leaves the major circle of the iris, decreasing rapidly in caliber, to terminate in an incomplete ring of arteriovenous anastomoses at the level of the collarette.31 These anastomoses represent the minor circle of the iris.30 From the minor circle, branches pass to the pupillary margin, forming continuous capillary arcades that join vessels located posterior to the sphincter muscle. By comparison, in the rabbit not all of the radial vessels originate from the major circle; instead, some of them represent direct continuations...
of long posterior ciliary arteries. The minor circle of the iris is not prominent in the rabbit, although a well-developed iridial circle is present at the pupillary margin. Ultrastructural studies of human eyes indicate that the major circle of the iris usually contains two layers of smooth muscle cells, whereas the radial iris vessels contain only one layer of smooth muscle cells. Saari reported that one layer of smooth muscle cells is found in the radial iris arterioles of the pig.

More recent studies on human eyes have challenged many of these basic tenets. Portions of the major circle of the iris have been reported to occasionally be present in the iris itself. Other authors have demonstrated that the major circle of the iris is not continuous and that the anterior ciliary arteries may not anastomose with the major circle at all but instead may contribute directly to the iris vasculature in addition to that of the episclera and the ciliary muscle. These findings are supported by recent angiographic studies in which the effects of tenotomy on iris circulation were examined. These studies concluded that the vertical anterior ciliary arteries were the predominant contributors to the iris circulation and that the major iris circle provided no appreciable collateral supply. In similar blood-flow studies on macaques and baboons it was reported that after complete tenotomy, blood flow to the anterior segment is decreased by 70% to 80%. This reduction suggested that the anterior ciliary arteries are the major contributors to the iris circulation in these species also and that the collateral flow from the major circle of the iris via the long posterior ciliary arteries is minimal. Our finding that the major circle of the iris in rhesus monkeys...
Fig. 6. Vessel near the anterior border layer of the iris in *M. mulatta*. In this region of the iris, characterized by a condensation of stromal cells, the vessel exhibits a thick, cellular adventitia composed of fibroblasts and melanocytes (M). A discontinuous row of lamellar processes belonging to pericytes is present (P). Two nerve processes are indicated by the arrows. (x 10,514.) Inset, Light micrograph of the same vessel demonstrating the thick, cellular adventitia and the relationship of the vessel to the anterior border layer (top). (x830.)

is discontinuous may be a significant factor in such flow reductions. Furthermore, the absence of a minor circle in monkeys suggests that the irides of these animals might be even less capable of collateral flow than is the human iris.

Iris vascular endothelia. The ultrastructure of the smallest iris vessels has been examined in several species. A nonfenestrated endothelium was reported in human, rabbit, pig, and guinea pig. In the rat, however, both nonfenestrated and fenestrated vessels were observed. In the cat a nonfenestrated endothelium was described, but in kittens fenestrae were seen.

In this study on rhesus monkeys as young as 6 months, we never encountered fenestrated vessels in the iris. It is known, however, that in rhesus monkey fetuses, fenestrated...
Fig. 7. Vessel posterior to the sphincter muscle of the iris in *M. mulatta*. In this region of the iris, in which stromal cells are more sparse than near the anterior border layer, a less complex adventitia is seen around the vessel. (×7470.) Inset, Light micrograph of the same vessel. The arrow points to the space that intervenes between the cellular portion of the adventitia and the collagen sheath characteristic of iris vessels. This iris sphincter muscle is seen above left and the posterior iris epithelium is below right. (×500.)

Transected vessels are constantly found at the root of the iris. Thus, only during prenatal life are fenestrated vessels found in the iris microvasculature of the rhesus monkey.

**Tunica media of iris vessels.** Disagreement exists regarding the tunica media of iris vessels. In a study of human iris, Ikui et al. reported that smooth muscle cells and elastic fibers were absent in all vessels of the iris, including those of large diameter. A "thin" tunica media was described by Tousimis and Fine and by Vegge and Ringvold, although the specific cell types were not mentioned. Hogan et al. have maintained that the radial iris vessels do contain smooth muscle cells within their media, but they do not mention the existence of pericytes around either larger vessels or capillaries. Many other researchers, however, have reported that pericytes are common in these vessels.
no smooth muscle cells are present in the lamina.

Our results demonstrate that in *M. mulatta*, no smooth muscle cells are present in the walls of iris vessels and that all iris vessels, regardless of their caliber, have the same structure. Their wall is composed of a continuous endothelium and basal lamina, surrounded by a discontinuous row of pericytes and a generally continuous adventitia of fibroblasts and melanocytes. These findings clearly demonstrate that vascular units composed of arteriole-capillary-venule (ACV), as typically found in other parts of the body such as the diaphragm and retina, cannot be identified in the iris microvasculature. The entire iridial vascular network is instead formed by vessels of different diameters but whose walls are ultrastructurally identical.

**Classification of iris vessels.** The criteria for electron microscopic identification of the various types of vessels that compose the microvasculature are based on studies performed primarily on subdermal vessels of rabbits and have recently been summarized by Baez. Diagnosis of the vessel's type rests on three criteria: (1) diameter, (2) position in the vascular tree, and (3) fine structure of the vessel wall in thin-sectioned specimens.

By these criteria, no iris vessel can be classified as an arteriole, collecting venule, or larger venule, since all these vessel types by definition contain a variable complement of smooth muscle cells in their walls. Iridial vessels, lacking smooth muscle cells in their walls, could belong only to the class of either capillaries or postcapillary venules. Capillaries are generally described as endothelial tubes devoid of smooth muscle cells, connecting the smallest arterioles to postcapillary venules, or as vessels 5 to 10 μm in diameter, consisting of an endothelium, basal lamina, and occasional pericytes. In the iris, only the smallest vessels fulfill both criteria of vessel size and ultrastructure required for the definition of capillaries. The term postcapillary venule is commonly used to define microvessels with a diameter of 8 to 30 μm formed by and as a continuation of two to four confluencing venous capillaries, with an increasing number of pericytes and "veil cells" (fibroblasts) as the lumen size increases. Clearly the iris does contain both large afferent and efferent vessels; however, many of the large vessels give origin to smaller vessels rather than originate from them. Thus the traditional criteria used in studies of vascular trees seem inadequate to classify the various components of the iridial vasculature. All rhesus monkey iris vessels possess walls with a simple structure, but only the smallest vessels are in the size range required for a strict classification as capillaries.

In conclusion, the iris vessels of *M. mulatta* are arranged to compose an unusual microcirculatory pattern in which vessels of quite different diameters possess morphologically identical walls. No smooth muscle cells are found in rhesus monkey iris vessels, and no ACV vascular units can be discerned in or dissected from the iris vascular network. This latter feature can explain the lack of a clear-cut filling pattern of arterioles, capillaries, and venules in angiograms of human irides and, during fluorescein drainage, the absence of a distinct venous phase as is typically seen in retinal angiograms.

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