Analysis of Choriocapillaris Flow Patterns by Continuous Laser-Targeted Angiography in Monkeys

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PURPOSE. To investigate choriocapillaris flow patterns and its segmental distribution in monkeys by continuous laser-targeted angiography (LTA).

METHODS. A slit lamp was modified to incorporate two kinds of lasers (argon and diode). Carboxylfluorescein (CF) was encapsulated in heat-sensitive liposomes and injected intravenously. Encapsulated CF was released locally by applying a continuous heat beam provided by the diode laser (810 nm). Video angiograms were generated with excitation illumination provided by the argon laser (488 and 514 nm), to observe selective images of the choriocapillaris.

RESULTS. Continuous application of the diode laser disclosed three distinct phases (filling, plateau, and draining) of fluorescent images of the choriocapillaris. In the plateau phase, a cluster of lobules fed by a common arteriole was uniformly illuminated. This defined area did not change in size while a continuous diode laser was applied to the same spot. Only in posterior regions did the angiograms demonstrate that during the filling and draining phases each lobule was filled from a central spot and drained along a peripheral ring, showing honeycomb flow patterns. In peripheral regions, large choroidal vessels as well as choriocapillaris were observed.

CONCLUSIONS. Continuous LTA demonstrated clusters of lobules fed by a common arteriole, and each cluster was found to be functionally independent. There were regional differences in choriocapillaris flow patterns, which suggests that the choriocapillaris provides a more highly efficient system of outflow in posterior regions than in peripheral regions. This modified LTA method appears to be useful in analyzing choroidal circulation in vivo.

Implanted distribution of the choroidal circulation. A review of the literature shows that a segmental distribution1:6–12 has been postulated by workers whose observations are based on in vivo occlusion in animals and humans, whereas a nonsegmental view1:3–17 has been derived primarily from postmortem injection studies in animals and humans. The disparity between these viewpoints may be an important clue in understanding the mechanism of choroidal diseases. Flower et al.3 compared in vivo choriocapillaris ICG images and choriocapillaris morphology of postmortem corrosion casts. They concluded that no correlation exists between the choriocapillaris plexus architecture revealed by the corrosion casts and the observed choriocapillaris lobular filling. Rather, the complex distribution of blood pressure gradients between the many interspersed arterioles and venules connected to it determines the blood flow patterns through the plexus.

Laser-targeted angiography (LTA) was developed by Zeimer et al.18–20 It entails encapsulating CF, a derivative of fluorescein, in heat-sensitive liposomes (lipid vesicles that can be disrupted by heat), injecting the liposomes intravenously, and then disrupting the liposomes with a mild, noncoagulating laser pulse through the pupil. When the dye is released from the liposomes with a local pulse of heat, it yields a bright fluorescent bolus that selectively highlights choroidal vessels as the bolus travels through the bloodstream. Observing this bolus as it travels from the arteries through the arterioles, cur in macular regions and that distinct vascular patterns are observed anatomically among the posterior pole, the equator, and the periphery.1 Although it has been speculated that choroidal circulation patterns may be different in macular and peripheral regions, few physiological studies have demonstrated this difference in vivo, primarily because it is difficult to visualize the choriocapillaris. Hayreh1 performed fluorescein fundus angiography (FA) by intracarotid injection of fluorescein and produced choriocapillaris flow images. Flower et al.3–4 performed high-speed indocyanine green (ICG) fluorescein angiography by using pulsed laser excitation and produced better definition of the choriocapillaris filling than had been achieved with continuous illumination. In both of these studies, the focus was on the chorocapillaris of the posterior pole, and the regional differences of the choriocapillaris flow patterns were not well described. Alm and Bill3 performed a study with labeled microspheres in monkeys and reported that the blood flow of the macular area is 6.5 mg/min per square millimeter, 5 to 10 times that of the equatorial and peripheral regions. Archer et al.5 analyzed rapid sequence photography during the early phases of FA to study normal human choroidal circulation. They reported that the choroidal arteries and choriocapillaris in the macular area fill more rapidly than elsewhere and that the resultant fluorescence is the densest in this region. They commented that the earlier filling of the choroidal vessels in the macular area is probably owing to the shorter course of the ciliary vessels to this area, and the denser fluorescence probably reflects the very thick capillary network there.

There has been considerable controversy about choroidal physiology with regard to the end arterial system and segmental distribution of the choroidal circulation. A review of the literature shows that a segmental distribution1:6–12 has been postulated by workers whose observations are based on in vivo occlusion in animals and humans, whereas a nonsegmental view1:3–17 has been derived primarily from postmortem injection studies in animals and humans. The disparity between these viewpoints may be an important clue in understanding the mechanism of choroidal diseases. Flower et al.3 compared in vivo choriocapillaris ICG images and choriocapillaris morphology of postmortem corrosion casts. They concluded that no correlation exists between the choriocapillaris plexus architecture revealed by the corrosion casts and the observed choriocapillaris lobular filling. Rather, the complex distribution of blood pressure gradients between the many interspersed arterioles and venules connected to it determines the blood flow patterns through the plexus.

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capillaries, and venules generates selective angiograms of the choriocapillaris.\textsuperscript{21-23} The images obtained by this method are totally different from conventional fluorescein angiograms (FAs) or indocyanine green angiograms (ICG), in that they are highly selective for the choroidal circulation and clearly show the dynamic filling of the choriocapillaris. This original method uses a pulsed argon\textsuperscript{21,22} or Nd:YAG\textsuperscript{23} laser beam to disrupt heat-sensitive liposomes.

In a previous study,\textsuperscript{24} we modified the original method and used a continuous diode laser beam at 810 nm to warm up the target tissue so that heat could penetrate deeply into the choroid. The continuous application of a heat beam allowed the observation of long-term movements of the dye front in rat choriocapillaris, and we concluded that the choroidal circulation in rats is territorial under normal conditions. In the present study, we applied this modified method to primates to analyze the physiological choroidal circulation and its segmental distribution and to compare the choriocapillaris flow patterns in macular and peripheral regions.

**Materials and Methods**

**Instrumentation**

We used the same modified slit lamp as described in our previous study\textsuperscript{24} (model SL-10L; Topcon Corporation, Tokyo, Japan). An argon laser beam at 488 (blue) and 514 (green) nm (Novus 2000; Coherent, Palo Alto, CA) is directed through a reflection mirror and used as a slit light source to excite fluorescent dye. To warm the target tissue, a diode laser (IR laser) beam at 810 nm (F-System; Coherent) is passed through a fiber optic cable and applied to the fundus through the slit lamp. A zoom system allows continuous variation of the diode laser spot diameter (50–1000 µm). In addition, an IR laser beam’s duration and power can be adjusted with an attached controller. Continuous IR laser application mode is installed and controlled by a foot switch. An IR laser spot can be moved with a joystick. Images taken by a CCD camera (Sony, Tokyo, Japan) mounted on the modified slit lamp are amplified by a video enhancer (Seprotec, Tokyo, Japan) and displayed on a monitor. Two kinds of filters are used. A long-pass filter (Omega Optical, Tokyo, Japan) passes any wavelength longer than 550 nm, and a short-pass filter (Omega Optical) passes any wavelength shorter than 620 nm. The wavelength range recorded by the CCD camera is 530 to 620 nm. These images are recorded on media with a digital video recorder (Sony) for analysis. The basic resolution of the recorded images from the digital video recorder is 47 pixels/mm, and 15 images are recorded per second. The sequences of the video images are analyzed on computer (Photoshop; Adobe Systems, Mountain View, CA).

**Measurement of Actual Laser Power**

The actual power of the IR laser at 810 nm and the illumination argon laser at 488 (blue) and 514 (green) nm were measured. The detector of a laser power analyzer (Ultima Labmaster; Coherent) was placed just in front of the cornea. The actual power of the illumination argon laser was 0.35 mW at the corneal surface (power density was 2.9 mW/cm²), and it was applied for up to 5.0 seconds. Under the National Standard for Safe Use of Lasers,\textsuperscript{25} a power of 0.35 mW of continuous argon laser is permissible. The measured intensity of the IR laser was found to be equal to that indicated on an IR laser beam controller. The IR laser was applied with a power of 20 mW for up to 5.0 seconds. The spot size of the IR laser was 250 to 500 µm in diameter (power density was 407 to 102 mW/mm²). The maximum energy density of the IR laser was 204 J/cm². According to the standard cited, a power of 20 mW (the power that we used) of continuous IR laser is considered class 3 in the Laser Hazard Classification of ANSI Standards.

**CF-Liposome Preparation**

6-Carboxyfluorescein (Molecular Probes, Junction City, OR) was purified on a hydrophobic gel, lipophilic column (Sephadex LH-20; Sigma-Aldrich, St. Louis, MO) and diluted to approximately 100 mM. The diluted dye was filtered through a 0.22-µm syringe filter (Millipore-GV; Millipore, Bedford, MA). Dipalmitoylphosphatidylcholine (DPPC) and dipalmitoylphosphatidylglycerol (DPPG; Genzyme, Liestal, Switzerland) were used without further purification. Liposomes were prepared by a method described by Mayer et al.\textsuperscript{26} Briefly, lipids were dried (dissolved in chloroform and methanol) to a thin film by rotary evaporation under vacuum. A 4% solution of CF filtered through a 0.22-µm filter was mixed with the dried lipid film, and the mixture was subjected to five freeze-thaw cycles. Next, extrusion sizing was performed in a thermobarrel extruder (Lipex Biomembranes, Vancouver, BC, Canada) through a stack of two 0.2-µm polycarbonate membranes (Millipore) 10 times, to yield large unilamellar vesicles. Unentrapped CF was removed by passing it through a column (Sephadex G-50; Pharmacia Biotech, Uppsala, Sweden). In the present study, 40°C liposome (phase-transition temperature, 40°C) was prepared by using DPPC and DPPG in a molar ratio of 4:1.

**Animal Preparation**

Two rhesus monkeys (Macaca fascicularis), weighing 2.5 to 4 kg each, were used. The animals were treated in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The monkeys were anesthetized with intramuscular ketamine (10 mg/kg) and xylazine (4 mg/kg). Topical 1% tropicamide and 2.5% phenylephrine hydrochloride were instilled for mydriasis during LTA. We experimented on four eyes. Each eye underwent several experiments during a course of 1 month. Before the experiment began, each eye was checked for normalcy by direct visual fundus examination.

**Laser-Targeted Angiography**

Immediately after 1.5 mL/kg of CF liposome suspension was injected intravenously through the tail vein of each animal, the laser beams were passed from the modified slit lamp through a lens (Super Macula 2.2; Folk, Mentor, OH) placed on the cornea to perform LTA. The IR laser with a diameter of 250 to 500 µm was applied to various locations of the fundus to release a bolus of dye from the liposomes. The IR laser energy was applied continuously up to 5 seconds. The IR laser application site was controlled with a joystick, and the laser power was adjusted to 20 mW. Argon laser at 488 (blue) and 514 (green) nm with a power of 2.9 mW/cm² was used as slit light to excite fluorescence. A long-pass filter (Omega Optical) was used to block any wavelength shorter than 550 nm. Because 514-nm argon laser light partially passes through the filter, dim background pseudofluorescent images of the fundus were observed without the presence of fluorescent material, facilitating focus and orientation on the fundus. Moreover, background fluorescence was caused by leakage of some CF (10%–15% of the dose) out of the liposomes due to their exposure to body temperature.

**Dye-Dilution Curve**

Dye-dilution curves were used to evaluate the hemodynamics of the choriocapillaris microcirculation. A circular area with a diameter of 28 pixels (200 µm; as seen through the CCD camera) was placed at a desired location inside a cluster of lobules that was illuminated by the argon laser. The location of the circle was 500 µm away from the IR laser application site. In selecting the circle location, we avoided areas where fluorescence from large vessels was observed. The average value of pixel light intensities within the circle was derived for each of the sequential images, and then normalized values were calculated by subtracting the background value from the average value, yielding a plot of the local fluorescence intensity as a function of time. The background value was produced by the combination of the pseudofluorescence and the leaked CF, as described before. The moment when the laser power was turned off was set as time 0, and the fluorescence intensity at time 0 was set as 100%.
Histopathology

In one animal, multiple dye releases were targeted in the foveal region three times over a course of 1 month. The IR laser was applied at a power of 20 mW for a total of 120 seconds. After the last experiment, the animal was killed with an overdose of pentobarbital sodium. The eye was enucleated, placed in fixative (phosphate-buffered 2.5% glutaraldehyde) for 24 hours, dehydrated with graded alcohols, and embedded in epoxy resin. Serial sections 2.5 μm thick were obtained from the treated area. The fovea was identified in the specimens to certify the location of laser exposure. Every fifth section was stained with hematoxylin-eosin and examined under a light microscope.

To determine whether the procedure caused temporary disturbance of the blood-retinal barrier, multiple deliveries were performed in the macular region for 30 seconds, followed by fluorescein angiography in the area exposed to targeted delivery.

RESULTS

Laser-Targeted Delivery

Three distinct phases of fluorescent images of the choriocapillaris were observed by applying the IR laser continuously for up to 5.0 seconds (Figs. 1, 2). The first was a dynamic filling phase, which was observed immediately after the start of laser application to the fundus. In the second, a plateau phase, the same area continued to fluoresce, no matter how long laser energy was applied to the same location. The third phase, a draining phase, began immediately after laser application stopped. The dynamic filling phase lasted approximately 0.8 to 1.0 second. The draining phase was approximately 1.0 to 2.0 seconds. The plateau phase was controlled by how long the IR laser was applied.

In posterior regions (Fig. 1), discrete spots of fluorescence away from the IR laser-delivery site appeared and expanded radially in the filling phase. Dye spread radially from each spot of fluorescence to the surrounding area of the choriocapillaris, and subsequently each dye front coalesced to illuminate a cluster of lobules uniformly. The dye filling was not pulsatile, but stable. In the plateau phase, a cluster of lobules was uniformly illuminated. In the draining phase, nonfluorescent blood flow entered from a central spot of each lobule, expanded radially, and drained along a peripheral ring, showing a honeycomb pattern.

In peripheral regions (Fig. 2), in contrast, fluorescence expanded from the IR laser-delivery site in the peripheral direction and fluorescence from large vessels and from the choriocapillaris was observed in the filling phase. In the plateau phase, a cluster of lobules was uniformly illuminated, and it was interlaced with bright fluorescence from large vessels. In the draining phase, a nonfluorescent area expanded from the laser site in the peripheral direction, and it did not show the honeycomb pattern that was observed in posterior regions.

In the plateau phase, in both the posterior and peripheral regions, a cluster of lobules was uniformly illuminated, stretching peripherally from the IR laser site (Figs. 1, 2). This illuminated cluster did not change in size during laser application. The dye did not diffuse beyond a border of the cluster area, and the cluster area continued to demonstrate a uniform degree of fluorescence in images (C) and (D) were practically the same (plateau phase). After the light from the laser was turned off at time 4.30, draining patterns were observed (E). In the draining phase, blood flow with fluorescent dye was replaced gradually by fresh blood flow without dye (F, black spots), showing a honeycomb pattern. In (F), the thick white line represents a borderline of a cluster of lobules fed by a common arteriole, whereas the thin white lines represent borderlines of lobules. Note that locations of white spots and black spots correspond well with each other.
fluorescence. A cluster of lobules in posterior regions is approximately the size of an optic disc, and its shape resembles a small maple leaf. A cluster of lobules in peripheral regions is larger than in posterior regions, stretching in the peripheral direction for a length of 3 to 4 disc diameters, and it is shaped like a flame of candlelight. We observed this cluster area as we moved the IR laser site to various proximate positions in both the posterior and peripheral regions (Figs. 3, 4). Laser application illuminated each corresponding cluster area of the choriocapillaris, and adjacent clusters fit with almost no gap or overlap, like pieces of a jigsaw puzzle. Figure 5 shows the locations of sites in Figures 1 to 4.

**Dye-Dilution Curve**

Choriocapillaris hemodynamics was evaluated by dye-dilution curves (Fig. 6). In the draining phase, we calculated the normalized light intensity by subtracting the background light intensity from the average light intensity within a circular area 200 μm in diameter. The circle was located 500 μm from the

**Figure 2.** Serial LTA images of normal choriocapillaris in equatorial regions (Fig. 5, area B). Retinal vessels (white arrowhead) demonstrated very weak fluorescence due to leakage of some CF from the liposomes caused by their exposure to body temperature. Light from the IR laser (white arrow), with a power of 20 mW, was aimed at an equatorial location outside arcade vessels (black arrowhead) and kept there for 4.50 seconds. Laser application started at time 0. Images were obtained at the following times (seconds): (A) 0.40, (B) 2.00, (C) 4.00, and (D) 4.70. In the filling phase (A), fluorescence expanded from the laser-delivery site in the peripheral direction (refer to Fig. 9). Note that fluorescent images (B) and (C) were practically the same (plateau phase). After the light from the laser was turned off at time 4.30, draining patterns were observed (D). In the draining phase, the nonfluorescent area expanded from the laser site in the peripheral direction. It did not show the honeycomb pattern that was observed in posterior regions. In equatorial regions, fluorescence from large vessels and from the choriocapillaris was observed (black arrow).

**Figure 3.** Comparison of four clusters of lobules that were illuminated in the plateau phase by applying a diode laser to four proximate positions in posterior regions. (A–D) Four different areas of the choriocapillaris were illuminated by aiming the laser light at four different proximate positions just temporal of the fovea. (E) Composite image generated by mapping the illuminated areas of (A), (C), and (D) and projecting them onto image (B). Note that all clusters were located side-by-side with almost no gap or overlap. Although the borderline of each cluster was highly distorted and complex, they fit like pieces of a jigsaw puzzle.
IR laser application site. We compared the dye-dilution curves obtained from posterior regions (four cases) and peripheral regions (four cases). The data are shown in Table 1. The average time to reach 50% of the fluorescence intensity level was 0.33 ± 0.04 second in posterior regions and 0.69 ± 0.07 second in peripheral regions. The data of these two groups were analyzed by the unpaired t-test. The difference between the two groups was considered statistically significant (P < 0.0001). It is suggested that fluorescent dye in the choriocapillaris was more quickly replaced by nonfluorescent blood in posterior regions than in peripheral regions.

**Histopathology**

Under light microscopy, no histologic abnormalities were observed in the choroid, RPE, or neurosensory retina in any of the prepared sections that were cut across the areas exposed to diode laser (Fig. 7). The fluorescein angiograms generated after multiple deliveries to evaluate the effect on the blood-retinal barrier demonstrated that the procedure did not cause leakage, as shown in Figure 8.

**DISCUSSION**

The development of LTA was first reported by Asrani et al.21,22 and Kiryu et al.23 and was applied to rats and primates. These investigators indicated that LTA had several advantages over conventional FA and ICG. First, the shadows of retinal vessels that are cast on the choroidal angiograms clearly indicate that the liposomes are effectively lysed in the choroid but remain intact in the retina. In the study of a noninvasive technique for monitoring chorioretinal temperature with a thermosensitive liposome, Miura et al.27 demonstrated that the IR laser energy necessary to raise the temperature of retinal vessels is higher than that needed to raise the temperature of choroidal vessels. This finding was thought to be due to the minimal absorption of the IR laser energy by the retina compared with its absorption in the RPE and the large amount of blood in the choroid. The lack of fluorescence in the retinal vessels ensured that the choroidal vasculature could be visualized separately. Second, the short-pulsed release, accompanied by rapid washout, ensured that the dye did not accumulate outside the vessels perfused by the bolus. Third, the angiograms could be repeated for at least 45 minutes.

In the original method, the authors used a modified fundus camera to which laser-delivery optics were added. They used a pulsed argon21,22 or Nd:YAG23 laser beam (200 ms) to heat liposomes and release the bolus of dye. In our previous experiment,24 we modified the original LTA method in two respects. First, we used a modified slit lamp because laser-delivery optics were more easily added to a slit lamp than to a fundus camera. Second, we used a continuous diode laser beam at 810 nm to warm up liposomes so that the heat could penetrate deeply into the choroidal tissue. These modifications allowed the observation of long-term movements of the dye front in rat choriocapillaris, and a cluster of lobules fed by a common arteriole was clearly demonstrated.

The most significant difference between primate fundus and rat fundus is that primate fundus includes the macula, a specialized area for vision where the circulatory supply from the choriocapillaris, not from retinal vessels, is dominant. It is known that in humans some choroidal diseases such as CNV predominantly occur in macular regions. Yoneya and Tso1 and Olver28 described regional anatomic variation in the angioarchitecture of the human choroid by using corrosion vascular

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**FIGURE 4.** Comparison of three clusters of lobules in equatorial regions. (A–C) Three different areas of the choriocapillaris were illuminated by aiming the laser light at three different proximate positions outside arcade vessels. (D) A composite image generated by mapping the illuminated areas of (A) and (C) and projecting them onto image (B). The clusters fit together like pieces of a jigsaw puzzle.

**FIGURE 5.** Schematic drawing of the indirect fundus image of the eye, from which the angiographic sequences in Figures 1–4 were derived. The boxed area A corresponds to the fundus area shown in Figures 1 and 3, and B corresponds to the area shown in Figures 2 and 4. X, location of the fovea.
casts and electron microscopy. Alm and Bill performed a study with labeled microspheres and reported that in monkey the blood flow of the macular area is 6.5 mg/min per square millimeter, 5 to 10 times that of the equatorial and peripheral regions. Although it is speculated that there may be a difference between choroidal circulation patterns in macular and peripheral regions, few physiological studies have demonstrated this difference in vivo, primarily because of the difficulty in visualizing the choriocapillaris. In our previous LTA study, regional variation of the choriocapillaris flow patterns was not observed in rats.

By applying LTA to primate eyes, we have demonstrated regional differences in choroidal circulation. First, choriocapillaris flow patterns were different between posterior regions (Fig. 1) and peripheral regions (Fig. 2). In posterior regions, the choriocapillaris was filled and drained in a honeycomb pattern. This pattern is represented schematically in Figure 9A. Lobules filled from central spots and drained along peripheral rings. In peripheral regions, the choriocapillaris was filled and drained in one direction, from the laser-delivery site to the periphery (Fig. 9B). This regional difference in choriocapillaris flow patterns was consistent with anatomic observations in corrosion vascular cast studies. Yoneya and Tso have reported that a lobular arrangement of the choriocapillaris is only seen at the posterior pole. At the equatorial area, the capillaries are arranged in a spindle-shaped segment. At the periphery, the choroidal arterioles and venules take a parallel course, with the choriocapillaris joining the adjacent arterioles and venules at right angles in a ladder pattern.

Second, while in posterior regions only the choriocapillaris was observed by LTA, in peripheral regions, fluorescence from large choroidal vessels and from the choriocapillaris was observed. This difference was also thought to be consistent with the anatomic observations that in posterior regions the choriocapillaris lies in a plane above the arteriolar and venous vessels that feed and drain it. In peripheral regions, the choroidal arterioles and venules were seen to take a parallel course with the choriocapillaris. We compared the dye-dilution curves of the choriocapillaris in posterior regions and peripheral regions (Table 1, Fig. 6). It seemed that the fluorescent dye in the choriocapillaris is more quickly replaced by nonfluorescent blood in posterior regions than in peripheral regions. This was thought to be because the circulation speed of the choriocapillaris is faster in posterior regions than in peripheral regions or the choriocapillaris circulation pathway is shorter in posterior regions than in peripheral regions. In either case, the lobular patterns in the posterior pole could provide a more highly efficient system of outflow than the spindle- or ladderlike patterns in the equatorial and peripheral choroids.

The extent to which the choroidal circulation is segmentally supplied or richly anastomotic has been the subject of much debate. Olver has described the functional anatomy of the normal choroidal circulation in humans from scanning electron microscopic examination of microvascular casts. He concluded that the choroidal vasculature has a distinct segmental arrangement, both at the level of the larger vessels and at the level of the smaller vessels (choroidal arterioles and choriocapillary lobules) and that there is a potential for acute choroidal ischemia such as triangular-shaped areas of choroidal filling delay on fluorescein angiography with variable overlying retinal dysfunction. In contrast, many studies have demonstrated potential collaterals from interarteriolar and interve-

![Figure 6](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932926/)  
*Figure 6. Dye-dilution curves of the choriocapillaris in posterior and peripheral regions. The moment the laser power was turned off was set as time 0, and the fluorescence intensity at time 0 was set as 100%. Normalized fluorescence intensity of the choriocapillaris was plotted as a function of time.*

**Table 1.** Time to Reach 50% of the Fluorescence Intensity Level in the Dye-Dilution Curves of the Posterior and Peripheral Regions

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<th>Posterior</th>
<th>Peripheral</th>
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<tr>
<td>Animal 1</td>
<td>0.34</td>
<td>0.60</td>
</tr>
<tr>
<td>Animal 2</td>
<td>0.38</td>
<td>0.70</td>
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<td>Mean ± SD</td>
<td>0.33 ± 0.04</td>
<td>0.69 ± 0.07</td>
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Data are expressed in seconds. The data from these two groups were analyzed by the unpaired t-test, and the difference between them was considered statistically significant (P < 0.0001).
nular anastomoses and the spontaneous recovery of acute choroidal ischemia.

With LTA, in the plateau phase, a cluster of lobules was uniformly illuminated, and this illuminated area did not change in size during laser application (Figs. 1, 2). We observed this illuminated area of fluorescence as we moved the IR laser site to various proximate positions (Figs. 3, 4). Laser application to each position created a corresponding cluster of lobules. Each cluster of lobules was located side by side with almost no gap or overlap. Based on this finding, each cluster of lobules was functionally independent and appeared to be fed by a common arteriole. Clusters of lobules that were uniformly illuminated in the plateau phase of LTA were consistently observed in all the experimented eyes. Each eye underwent multiple experiments through the course of 1 month and demonstrated no temporal difference of the dye filling patterns.

We considered the effects that IR laser heating of interstitial tissues could have on choriocapillaris blood flow. The heating effects were localized in the IR laser application site, with no influence over the area where the released fluorescent material really flows down. Other than these localized heating effects, the conditions in which the segmental filling patterns of clusters of lobules were observed were considered to be near physiological. In contrast, pathologic conditions such as obstructed arteries or veins and high intraocular pressure could change these patterns, particularly if the territorial circulation is due to the working pressure gradient within it. These observations were in agreement with previous reports. Flower et al.3 compared in vivo choriocapillaris ICG images and choriocapillaris morphology of postmortem corrosion casts. They concluded that no correlation exists between the choriocapillaris plexus architecture revealed by the corrosion casts and the observed choriocapillaris lobular filling and that choriocapillaris filling patterns are determined by the network of perfusion pressure gradients that exist among the interspersed feeding arterioles and draining venules connected to the choriocapillaris plexus. Olver28 reported that although vascular casting supports the concept that choroidal vasculature has a distinct segmental arrangement, the choroidal circulation is

FIGURE 7. Light micrograph at the LTA site. The section was taken in an area next to the fovea exposed with 20-mW diode laser light for up to 120 seconds. No significant sign of retinal or choroidal damage is observed. Hematoxylin-eosin; original magnification: (A) × 200; (B) ×400.

FIGURE 8. Fluorescein angiogram of a macular area. After a total of 30 seconds of IR laser application to this area for multiple deliveries of dye, the routine angiogram was obtained 5 minutes after fluorescein injection. The area shown is from the same eye as shown in Figures 1 and 3. Note the absence of staining and leakage, indicating that the blood-retinal barrier was not affected, even temporarily.
not truly endarterial, since there are potential collaterals from interarteriolar and -venular anastomoses.

There is a strong similarity between the clusters of lobules illuminated in the plateau phase by LTA and the three-sided appearance of acute triangular syndromes, which are caused by acute choroidal ischemia due to thrombosis in the posterior ciliary arteries (PCAs) or their arborizations.\textsuperscript{29,50} It is thought that in LTA the IR laser warms a choroidal arteriole and the released fluorescein flows downstream to the area of the choriocapillaris fed by the same arteriole. Although Asrani et al.\textsuperscript{21,22} in their original method used the argon laser to cause the release of a bolus of dye from liposomes, we used the IR laser at 810 nm to warm up target tissue so that heat could penetrate more deeply into the choroidal tissue. We believe that heat created by the IR laser penetrate into choroidal arterioles and releases a bolus of dye.

The argon laser used to illuminate the fundus is within the level judged permissible according to national standards for use in humans.\textsuperscript{23} The power of the continuous IR laser to release dye in the choroid is considered class 3 of Laser Hazard Classification of ANSI standard. To study the effects of the IR laser and the argon laser on the permeability of the microvascular structures, we performed FA (Fig. 8). Multiple dye releases were targeted in the macular region, fluorescein was injected 30 minutes after the releases, and angiograms were obtained 5 minutes after injection. There was no staining or leakage in the treated area, indicating that the blood-retinal barrier was not affected, even temporarily. In the original method described by Kiryu et al.,\textsuperscript{23} the IR laser was applied for less than a second to release the dye in the choroid, and its power level was permissible according to ANSI standard. Because we used the continuous IR laser up to 5 seconds, the power level that was considered class 3 of Laser Hazard Classification of ANSI standard was necessary. According to Miura et al.,\textsuperscript{27} 20 mW of the IR laser could raise the temperature in the normal choroid up to 48°C. However, their data could not be easily compared with ours because their data were derived from rats. To study the safety of the continuous IR laser, we obtained histologic samples from one eye in which multiple dye releases were targeted in the foveal region (Fig. 7). To see the latent heat-related damage, the same eye was subjected to experiments three times over a course of 1 month before the animal was killed. Under light microscopy, no histologic abnormalities were observed in the choroid, RPE, or neurosensory retina.

The quenching phenomenon of fluorescence is vital to understanding the mechanism of LTA. Heat-sensitive liposomes encapsulating CF (CF liposomes) are vesicles of lipid bilayers. Although CF is encapsulated inside the liposome, there is little fluorescence. This is due to fluorescence quenching at high CF concentrations.\textsuperscript{18,19} In contrast, where the laser is applied, CF is released and fluoresces strongly after its dilution in the serum. Because of the quenching phenomenon, the targeted illumination of vessels and the analysis of dye dilution curves are possible in LTA.

Our modified LTA method was a powerful tool in the analysis of the choroidal circulation in vivo. It has provided evidence that the choroidal circulation is territorial under normal conditions and that the choriocapillaris flow patterns are different between posterior and peripheral regions.

References