Retinal Microvascular Surgery: A Feasibility Study

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PURPOSE. To evaluate the feasibility of microvascular surgery at the level of the retinal vasculature.

METHOD. Porcine eyes were used, and eyecups were prepared under an operating microscope. Several classic microvascular maneuvers were explored, such as vascular puncturing, catheterization, mobilization, intravascular injections, and various combinations of the same. Instruments used ranged from disposable 30-gauge needles to an Er:YAG laser. Commercially available 10-0 nylon sutures, fine polyimide tubes, and custom-made fine glass tubes were used for vascular catheterization.

RESULTS. Puncturing, mobilization, catheterization, and intravascular injection of retinal arteries and veins were possible. The connection of two remote retinal vessels with a fine tube was achieved with the combination of these maneuvers.

CONCLUSIONS. The feasibility of performing several microvascular maneuvers on retinal arteries and veins was demonstrated in porcine eyes. Further experimentation and development of these findings in a living animal model could lead to the application of such microvascular maneuvers in humans.

In retinal vascular surgery is emerging as an exciting area of microsurgical intervention in the posterior segment. The recent development of sheathotomy, chorioretinal anastomosis, and retinal intravascular injections represent exciting new potential interventions, and ongoing clinical trials will help define their utility. Another new frontier has been described in recent publications of novel surgical approaches to retinal vascularization being developed in cadaveric eyes and animal models. Although retinal vasculature surgery presents new challenges in extremes of size, tissue delicacy, and constraints in approach, the same basic principles of macrovascular surgery used in fields such as hand and cardiothoracic surgery can be brought to bear. In the present study, we explored these basic steps—namely, vascular localization, mobilization, creation of vascular openings, cannulation, and reanastomosis—as applied to retinal vascular surgery and the potential of combining these steps into effective therapeutic procedures.

MATERIALS AND METHODS

Porcine Eyes

More than 80 porcine eyes were used in the study. The eyes were delivered fresh and were used within 24 hours of enucleation. Eyes were stabilized in a Styrofoam mount under an operating microscope (Wild M651; Wild Leitz USA, Inc., Rockleigh, NJ). The anterior segment was excised by circumferential incision at the level of the pars plana. The vitreous base was massaged with a cotton-tipped applicator, which caused separation of the vitreous base, making it possible to remove and “roll” the vitreous en bloc out of the eye with the dry cotton-tipped applicator. This en bloc method allowed removal of vitreous in its entirety with minimal traction on the retina. A blunt 30-gauge cannula was then used to aspirate the residual fluid from the retinal surface. All maneuvers were performed on the eyecup in room air.

Preparation of Glass Tubes

Fine glass tubes were prepared with standard glass pipettes heated with a Bunsen burner and pulled quickly in a single, smooth motion. Glass tubes with a patent lumen could be formed in this manner with an outside diameter equivalent to 40- to 42-gauge.

Vascular Puncture

Puncturing of retinal arteries and veins was performed with a variety of sharp instruments, including bent MVR blades, suture needles (CS 160 Ultima needle; Ethicon, Inc., Somerville, NJ), and bent 30-gauge needles (B-D 30G1⁄2; BD Biosciences, Franklin Lakes, NJ). The end point was the creation of an opening in the vascular wall of sufficient size to allow passage of a tube or cannula. The combination of Er:YAG laser ablation of the vascular wall followed by mechanical puncturing was also tested.

Vascular Catheterization

Catheterization of arteries and veins was performed after vessel wall puncture. Glass tubes, 10-0 nylon sutures (black monofilament nylon suture; Ethicon, Inc.), polyimide tubes (50.8-μm internal diameter and 7.6-μm wall thickness; HV Technologies, Trenton, GA), and subretinal cannulas (model 5221 [Lambert]; Visitec, Sarasota, FL) were used. A bevel was created at the end of both the glass and the polyimide tubes for easier vascular access. At the end point was the advancement of the catheterization device into the vessel to a length that would allow for vessel-to-vessel anastomosis, and/or intravascular injections.

Intravascular Injection

Intravenous injections were achieved with a curved subretinal cannula with a 230-μm tip (5221 Subretinal Cannula [Lambert]; Visitec). The cannula was attached to a 3-mL syringe, and a vein branch near the optic nerve was catheterized after it had been punctured. After successful catheterization, air, methylcellulose 2.5% (Gonak; Alcon, Inc., Buffalo Grove, IL), or a mixture of methylene blue 1 mg/mL (Sigma-Aldrich) and methylcellulose 2.5% was injected into the vein. The end point was advancement of the injection solution to the periphery with minimal macroscopic damage to the vessel.

Vascular Mobilization

Separation of the retinal vessels from the retinal surface and mobilization was achieved with bent 30-g needles (B-D 30G1⁄2; BD Biosciences), bent MVR blades, and Sutherland fine curved scissors (Alcon, Irvine, CA). The end point was the detachment of a vascular branch from the retinal surface and its mobilization to another retinal area with the least retinal trauma possible. In several cases, at the end of the procedure, methylcellulose was injected under the retina from a site remote to that of mobilization, and the retinal area where the vessel originally lay was excised by circumferential incision at the level of the pars plana.
was elevated. This allowed the extent of retinal damage to be evaluated directly by examining carefully for any passage of methylcellulose out of the subretinal space that would indicate a full-thickness retinal break.

Staining

Staining of the eyecup was accomplished by using methylene blue 1 mg/mL (Sigma-Aldrich) and was used as an adjuvant to improve visualizalization. For staining, one or several drops of methylene blue were placed on the retinal surface before vascular manipulations.

Er:YAG Laser

A solid-state Er:YAG laser (VersPulse Select Erbium; Coherent, Inc., Palo Alto, CA), operating at a wavelength of 2.94 μm with incremental energy levels ranging from 0.2 to 5.0 mJ per pulse, a pulse length of 300 μs, and repetition rates from 2 to 50 Hz, was used. The laser output was coupled to a flexible fiberoptic 2 m in length (Coherent, Inc.) that was connected to a 20-gauge endprobe ending with a straight 100-μm tip. The Er:YAG laser was used for preparing the arterial wall before puncturing with a sharp device. Energy of 1 to 2 mJ per pulse, pulse length of 300 μs and repetition rate of 10 Hz was used. Repetitive sets of a few pulses were applied to the arterial wall. The end point was thinning of the arterial wall to the point of a small herniation of the inner wall layers.

RESULTS

Vascular Puncturing

This maneuver was performed in 20 porcine eyes. Puncturing of veins was easily achieved with a bent 30-gauge needle or a fine suture needle. Sites near the nerve head, where the vein had a maximum diameter and was tightly tethered to the optic nerve head, were the easiest to puncture, but it was possible to puncture even quite distal from this area. Puncturing always led to retrograde blood flow and resulted in the creation of an opening of sufficient size (Fig. 1A). Direct puncturing of the arteries with a sharp instrument was more difficult. The arterial wall was smaller than that of the veins and tended to slide under the pressure of the sharp instrument. Puncturing became much easier after pretreatment of the puncture site with the Er:YAG laser. This laser resulted in superficial ablation of the vascular wall and the adjacent retina. An initial dilatation of the arterial wall was followed by a protrusion of the inner wall layers. When this occurred, the herniated layers were punctured mechanically. This series of maneuvers permitted a controlled puncture of the arterial wall in most cases (Fig. 1B). Puncture always led to retrograde blood movement.

Intravascular Injections

A curved subretinal cannula was used for intravenous injections. An opening in the vein wall was created near the optic nerve head, using the technique just described, and the cannula tip was inserted into the opening. If the tip was not maintained in the vein, or if a mixture (air, methylcellulose, or methylene blue/methylcellulose) was not injected, the vein would typically collapse, and repeated introduction of the cannula tip was difficult. However, with attention to these details, the cannula tip was easily advanced into the vein and its branches (Fig. 1A). In total, we performed this maneuver in 11 porcine eyes.

Vascular Mobilization

Mobilization of the retinal vessels was performed in 21 porcine eyes. Mobilization was achieved after separation of the vessel from the retinal surface and severance of its small side branches. To separate a vessel from the retinal surface, either a bent 30-gauge needle or one blade of the Sutherland fine curved scissors was carefully inserted under the vessel. Care was taken to stay as close as possible to the vessel wall, thus minimizing retinal damage by controlling the depth of insertion into the retina tissue. After the needle had passed under the vessel and its tip had emerged from the other side, slight vertical force was exercised to elevate the vessel. Then, with small horizontal movements the area of separation was gradually increased. Small side vessels were cut with scissors (Figs. 2A, 2B).

Mobilization of arteries was possible with only minimal, superficial retinal damage. A partial-thickness retinal lesion was usually induced, and only rarely was there a creation of a full-thickness retinal hole.

Mobilization of retinal veins was more demanding. The veins were usually deeper in the retinal tissue than the arteries. As a result, it was necessary to handle the tissue very gently to avoid a full-thickness retinal break. The thin venous wall and its tendency to collapse during manipulation represented an additional problem during vein mobilization. Rarely, the vein was punctured before mobilization, and a small amount of methylcellulose was injected into the vessel. The viscous material served as an intravascular support and facilitated the manipu-
tissue with greater ease than the curved needle, and the degree of tissue drag was substantially reduced.

**Catheterization**

Catheterization was performed after puncture of retinal veins and arteries with the techniques described earlier. A pair of fine forceps was used for grasping and handling the catheterization device. The main limiting parameter for vascular catheterization was the relation of the vessel diameter to the diameter of the catheterization device. Catheterization of both arteries and veins was relatively easy with a 10-0 nylon suture (Fig. 3A). Glass tubes were easily advanced into veins (Fig. 3B), but arterial catheterization was more demanding. The best catheterization results were achieved with polyimide tubing of 50 μm diameter. With these tubes, we were able to catheterize both veins and arteries of suitable size (Fig. 3C). Catheterization maneuvers were performed in a total of 28 porcine eyes.

**Staining**

Staining with methylene blue was used as a means to visualize retinal vessels after their lumens became bloodless and thus invisible. The dye tended to stain the retinal tissue more intensely than the vessels, allowing easy visualization of the vascular tree. Staining was used in 16 porcine eyes.

**Double Puncturing and Suture Insertion**

In three porcine eyes, puncturing of an artery at two sites several millimeters apart was performed as described earlier. A piece of 10-0 nylon suture was prepared. Each end of the suture segment was inserted into a separate arterial wall opening (Fig. 3A), resulting in a simulated "bypass" of the vascular segment between the two openings.

**Mobilization for Anastomosis**

Mobilization and severance of retinal arteries was performed in an effort to achieve configurations which would facilitate anastomosis of different vessels. In one case, two adjacent vessels were mobilized and placed side by side in an arrangement that would presage a side-to-side anastomosis (Fig. 2A). Vessels were also mobilized and cut and their ends were placed in apposition to each other in an arrangement leading to an end-to-end anastomosis (Fig. 2B). Maneuvers to achieve arrangements that would facilitate vascular anastomosis were performed in six eyes.

**Vascular Anastomosis with a Glass Tube**

Puncturing of two arteries was performed with the technique described earlier. A curved glass tube was prepared. With a pair of fine forceps, each end was inserted into the lumen of a punctured vessel. In this way, an anastomosis of the two vessels was achieved with the glass tube (Fig. 4). This maneuver was performed in three eyes.

**Redirection of Vascular Route with a Polyimide Tube**

In 10 porcine eyes, a combination of maneuvers was tested aiming at redirection of the vascular route with a polyimide tube. The wall of an artery was punctured, and the vessel was catheterized with the piece of tubing. After catheterization, the vessel was mobilized along its entire length from the catheterization site to the optic nerve. All side branches were meticulously cut during the mobilization process. The vessel was then cut just distal to the catheterization site. This left the proximal portion of the vessel separated from the retinal surface and catheterized with the polyimide tubing at its distal end (Fig. 5A). A second artery in a different position was then punctured.
tured. The second vessel was selected in a location that could be approached by the first mobilized vessel. The extravascular portion of the polyimide tubing was grasped with a pair of fine forceps and inserted into the second vessel. In this manner, we achieved redirection of the route of the proximal segment of the first vessel toward the distal segment of the second vessel (Fig. 5B).

**DISCUSSION**

The importance of retinal vascular manipulation for the treatment of vasculopathies has received increasing attention during recent years. To date, however, only minimal experimental or clinical interventions such as puncturing, probing, and sheathotomy have been tried.1-7 In the present study, we have demonstrated the feasibility of additional surgical maneuvers on retinal vessels in a manner analogous to vascular surgery elsewhere in the body. Using a step-by-step approach in a porcine eye model, we have shown that several basic maneuvers can be combined for the performance of more complex manipulations.

Intravascular access, probing, and catheterization were the first maneuvers that were investigated. We were able to duplicate the findings of previous researchers who have shown that puncturing, probing, and intravenous injections can be performed in retinal vessels.5-8 For arterial puncture, we found that pretreatment with the Er:YAG laser, to thin the tougher arterial wall, allowed subsequent puncture with a sharp instrument to be easily and reliably achieved. Conversely, the handling of the relatively more compliant veins was facilitated by the intravascular injection of a supporting material. In addition to venous injections of air and aqueous solutions, we were also able to inject a viscous material that provided internal support for the thin-walled veins in our porcine eye model and facilitated their separation from the retinal tissue and mobilization, although this could be accomplished with more difficulty without injection.

Probing and catheterization of the vascular lumen was achieved with sutures, glass tubes, and polyimide tubes. The end point in this study was the advancement of the catheterization device into the vascular lumen for a sufficient length and not the mere insertion of its tip into the lumen. Beveling of the catheterization tube tip was critical for easing entry. The most important factor affecting the ease of vascular catheterization was the catheter diameter. We achieved the best results with polyimide tubes with an internal diameter of 50.8 μm and a wall thickness of 7.6 μm. With these, we were able to catheterize both veins and arteries. This size seems to represent the maximum catheter diameter that can be used for...
arterial catheterization. Because porcine and human retinal arterioles share similar sizes, these findings can be used as a reference for future experiments in humans. Taking into account that the average luminal diameter of the large human retinal arteries is 120 μm and decreases to 8 to 15 μm in the periphery, it is obvious that tubes of smaller diameter must be used to catheterize peripheral arteries. Although microcannulation of both arteries and veins with fine tubes has been reported in the past, the advancement of a tube into a vessel has not been described in the literature, as far as we know. In their significant work concerning surgical approaches to retinal vascular occlusions, Tang and Han used only suture material as an experimental probing device. They stressed, however, the importance of retinal vessel cannulation with a catheter for the delivery of thrombolytic agents. A number of other possible applications of these basic maneuvers have been described in the literature, including measurement of retinal circulation pressures, and analysis of local vascular parameters such as metabolites and volume flow rates. Intravascular procedures using the Er:YAG laser after the development of appropriately sized laser probes might be added to this list.

The application of well-established macrovascular procedures, such as anastomosis and vessel bypass, to the microvascular level required by retinal vessels will necessitate the performance of more invasive maneuvers. Dissection and isolation of the vessels from the surrounding tissue represents the first prerequisite step. In this study, we were able to demonstrate that separation of retinal vessels from the retinal tissue can be achieved. Separation of retinal vessels from the retinal surface, known as retinal vascular avulsion, is known to happen automatically as a result of traction forces applied on the vessels. Similarly, surgical separation of retinal arteries from the retina is used as part of the newly developing sheathotomy surgery for branch retinal vein occlusions. We were able to achieve a controlled separation of retinal arteries from the retina to a considerable extent, in many cases up to several millimeters in length. This length, after severance of small branch vessels, is sufficient to allow for unconstrained mobilization of major arterial branches. Arterial separation and mobilization were achieved with minimal damage to the underlying retinal tissue, whereas venous mobilization was much more difficult because of their friable nature and deeper location. The intravenous injection of viscous material facilitated vein mobilization, but full-thickness retinal damage was more common, especially early in the experiments.

The connection of vascular branches represents another significant step for the completion of a major microvascular procedure. With combined mobilization of two arterial branches, we were able to arrange the vessels in a way that would facilitate a side-to-side or an end-to-end anastomosis. In addition, we were able to connect different vascular branches with glass or polyimide tubes. The combination of puncture, catheterization, and mobilization permitted the establishment of a connection similar to an end-to-end anastomosis. The demonstration of feasibility of vascular branch connection by means of fine tubes advanced into the vascular lumen may offer the basis for utilization of synthetic grafts in retinal microvascular surgery, although several questions regarding anastomosis functionality remain. In this work, we did not check the patency of the anastomosis achieved with the tubes. Moreover, no measures were taken to obtain a seal connection between the vessels and the tubes. In future experiments, these questions should be resolved and the patency of the anastomosis should be verified with dye injection. However, even if immediate patency is demonstrated, the long-term patency of small-caliber synthetic grafts remains a problem.

Other methods of vascular connection may also have a role in retinal vascular anastomosis. Although suturing may not be feasible because of size restrictions, the use of glue materials, such as cyanoacrylate or thrombin glue, may be useful alternatives. These glues have been used for microvascular anastomosis in other body locations, in addition to some work in retinal tissues such as gluing peripheral or macular holes.

In the current work, we used a porcine eye model and an open-sky approach. In so doing, we eliminated two major sources of difficulty. The first was the active blood flow of a living, retinal circulation. Management of the blood flow during retinal vascular procedures in the living eye represents a serious challenge. Tang and Han reported that hemostasis could be accomplished by simply increasing the intraocular pressure after penetration of the vascular wall in a living animal. They also proposed a bimanual technique and intravitreal injection of perfluorocarbon liquids to achieve hemostasis. Development of miniature vascular clips or “sandbags” could represent another option for hemostasis during retinal microvascular procedures. The second source of difficulty in future animal experiments is the added level of difficulty if vascular manipulations are performed through the small openings used in standard three-port pars plana vitrectomy. The development of specialized vitreoretinal instruments and the appropriate modification of some of these maneuvers are needed to overcome these hurdles.
In conclusion, we have demonstrated the feasibility of several microsurgical maneuvers on retinal vessels. Further experimentation and innovations in a living animal model are necessary to establish the feasibility of these maneuvers in the living eye. Classic microvascular techniques have been used with great success to reperfuse ischemic tissues in other fields, such as cardiothoracic and vascular surgery. Our results represent further steps toward the application of such classic microvascular techniques that could lead to vascular bypass and anastomosis at the level of retinal vasculature and the exciting promise of novel treatments for retinal vascular diseases.

References


