Dye Injection Method for the Demonstration of Territories Supplied by Individual Perforating Arteries of the Posterior Communicating Artery in the Dog

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SUMMARY Various reported methods to demonstrate the territory supplied by an intracranial artery are designed to deal with major cerebral arteries or a group of perforating arteries. We have devised a technique to demonstrate the area supplied by individual small perforating arteries using different dyes with gelatin. A temporal craniectomy was made in the dog and the posterior circle of Willis was exposed via a subtemporal route under the operating microscope. After a portion of the posterior communicating artery including one perforating artery was trapped with clips, dye was injected through the cannula. The animals were immediately sacrificed and studied. The area supplied by an individual perforating artery occupied a relatively large and distinct area, and when two dyes for two different perforators were used, no significant overlapping of the stained territories was seen. This method is useful for the study of focal cerebral ischemia caused by occlusion of a fine perforating artery and in particular it will serve to clarify the importance of preserving each perforating artery in surgery in the region of the base of the brain such as that for basilar artery aneurysm.

WITH RECENT ADVANCES in neurology, neurosurgery and neuroradiology, it has become increasingly important to know about the territory supplied by an individual perforating artery. Various methods including injections of dye, plastic materials, and fluorescein or radioopaque media, have been reported to study the territory supplied by a major intracranial artery in different species. All these procedures are, however, designed to demonstrate the territory supplied by a major cerebral artery or by a group of perforating arteries. We report a dye-injection method to demonstrate the areas supplied by individual perforating arteries from the posterior communicating artery in the dog.

Materials and Methods

Twenty adult mongrel dogs were used for the study. Under intravenous anesthesia with Pentobarbital (30 mg/kg), the head of the animal was placed and fixed in the dog skull frame, and a generous unilateral temporal craniectomy was made. An approach was made via a subtemporal route to expose microsurgically the ipsilateral internal carotid, posterior communicating artery and proximal posterior cerebral artery; special care was taken to preserve perforating arteries from these major vessels. There were usually two types of perforating artery from the posterior communicating artery: small arteries less than 0.3 mm in diameter and large ones greater than 0.5 mm. In this study, large perforating arteries from the posterior communicating artery were selected for injection of the dye. All smaller perforators were coagulated before injection of the dye. Usually two or three large arteries were found; however, only two arteriess were selected for dye injection: one was the perforator most proximal to the internal carotid artery (proximal perforator) and the other was the one closest to the posterior cerebral artery (distal perforator). When there were three large perforators, the middle one was coagulated and not used for dye injection. Four animals were used for each of the following procedures:

1. Injection of the dye into the proximal and distal perforators of the posterior communicating artery.

a. The proximal perforator

The proximal perforator was trapped by three Sugita clips (Mizuho Ika-Kogyo Co., Tokyo, Japan): two were applied to the internal carotid artery just proximal and distal to the junction with the posterior communicating artery, and the third clip to the posterior communicating artery just distal to the proximal perforator. After thus isolating the proximal perforator from the systemic circulation, 0.2 ml of 5% Carmin-red with 50% gelatin was injected through a 27½ gauge needle which was inserted in the trapped position of the internal carotid artery and connected to a 1 ml tuberculin syringe using an extension tube with an internal diameter of 1.1 mm. The dye was manually injected over a period of approximately 10 seconds with a pressure that did not cause excessive dilation of the vessel. After the injection was completed, the injected perforator was electrocoagulated with bipolar forceps and sectioned to prevent washing out of the dye.

b. The distal perforator

The distal perforator was trapped and isolated from the systemic circulation by three Sugita clips: two clips were applied to the posterior cerebral artery just proximal and distal to the junction with the posterior communicating artery and the third clip was applied to the posterior communicating artery just proximal to the distal perforator. Five percent Berlin blue 0.2 ml with 50% gelatin was injected in the trapped portion of the posterior communicating artery using the same tech-
niques as in the preceding procedure. After the injection, the injected distal perforator was coagulated and sectioned.

c. The proximal and distal perforators

Following the injection in the proximal perforator, the distal perforator was injected in the same animal as above.

2. Injection of the dye into the common carotid artery with the contralateral posterior communicating artery trapped.

The bilateral common carotid arteries were exposed in the neck and the unilateral temporal craniectomy was made. The posterior communicating artery including all its perforating branches was trapped by applying two Sugita clips to its proximal and distal ends. Fifty ml of 5% India ink with 50% gelatin was injected through the exposed contralateral common carotid artery. The ipsilateral common carotid artery was ligated prior to injection of the dye.

3. Combined injection of three different dies into two individual perforators of the posterior communicating artery and the contralateral common carotid artery.

The bilateral common carotid arteries having been exposed in the neck the unilateral temporal craniectomy was made. The subtemporal approach was made as described above, exposing the post. com. artery and its branches. Carmin-red and Berlin blue solutions were injected according to the procedures in 1. The entire post. com. artery was coagulated and the clips on the posterior cerebral artery were released. Immediately afterwards, India ink solution was injected, as in 2 above, into the contralateral common carotid artery.

The animals in each procedure were immediately sacrificed with intravenous injection of 10 ml of 15 w/v \% potassium chloride solution. The brain was removed, put in an ice-water bath for 30 minutes and then fixed with 10% formalin solution for 2 days. Two of 4 brains used for each procedure were serially sectioned coronally at 1 mm thickness and the other 2 brains were serially sectioned sagittally at 5 mm thickness. Anatomical distribution of the dyes in relation to the perforating arteries was observed and photographed.

Results

1. Injection in the proximal and distal perforators of the posterior communicating artery

Areas stained with either red or blue dye were relatively constant in location with some variation according to the animal and the number of major perforators. Areas stained red after injection of the dye into the proximal perforating artery were mainly the medial nuclear group of the thalamus. The area was larger rostrally than caudally. It never extended into the internal capsule. The subthalamic nuclei were always spared. The areas stained blue after injection of the dye into the distal perforating artery were the lateral and caudal thalamus, subthalamic area and posterior perforating substance. The stained areas were sharply demarcated without diffusion into the surrounding areas. When two dyes were injected consecutively in the two different perforators, the stained areas roughly corresponded to the areas stained individually. Overlapping of the dyes was minimal (fig. 1 A,B).

2. Injection of India ink in the common carotid artery with the contralateral posterior communicating artery trapped

Structures unstained with India ink were the ipsilateral thalamus, subthalamic area and part of the internal capsule. The unstained area extended caudally as far down as the ventral portion of the midbrain. The ipsilateral subthalamic area was always spared, although the degree of involvement of the internal capsule and the midbrain varied slightly. The anterior perforating substance was never involved. The margin of the area was sharp without diffusion of the dye.

3. Combined injections into individual perforators of the posterior communicating artery and the ipsilateral common carotid artery

The areas stained with three different dyes corresponded to the areas stained with respective injections. There was no significant overlapping of the dyes nor any diffusion.

Discussion

Duret described in 1874 a technique of staining the territory of a major cerebral artery using India ink with gelatin. This technique has since been used by many

![Figure 1. Consecutive injection of two different dyes into the proximal and distal perforators of the posterior communicating artery of a dog. A and B: cross section at the pre-mammillary plane and sagittal sections at 0.5 cm lateral to the mid-line of an injected brain, respectively. Note that red dye, injected in the proximal perforator, stains the medial and anterior portion of the thalamus, while blue dye, injected in the distal perforator, occupies the lateral and posterior portion of the thalamus and subthalamic area. Bars measure 5 mm.](http://stroke.ahajournals.org/)

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other authors. Beevor used 4 different dyes to demonstrate territories of major cerebral arteries simultaneously. Other methods that have been used include injection of materials such as plastics and barium. All these procedures are designed to demonstrate the territory supplied by a major cerebral artery or by a group of perforating arteries. The method used in the present paper is to demonstrate the territory supplied by individual perforating arteries using one or a number of dyes with gelatin. Studying individual perforating arteries is particularly important in the era of microcerebrovascular surgery when fine surgical procedures in the region of the base of the brain such as surgery for basilar artery aneurysm have become feasible. It is also important to study the artery responsible for a small infarct shown on a computer tomograph.

Our method consists of the isolation of an individual perforating artery by clipping and/or electrocoagulation, followed by injection of the dye. All procedures required the operating microscope to minimize surgical trauma to the brain. Microneurosurgical techniques made possible fine methods such as injection of the dye into the small posterior communicating artery of the dog with a diameter less than 1 mm. Regarding the pressure of injection, Tousant indicated in his experiment with the human cadaver that the pressure should not exceed 150 cm H2O when injecting micro-opaque solution. With our manual injection using a tuberculin syringe, although the precise pressure was not measured, we did not observe any significant difference between the territory made by injection into the posterior communicating artery and that made by injection into the common carotid artery after occluding the posterior communicating artery. With the experiment using different dyes for different perforators, there was also no significant overlapping of the dyes.

Our results showed that an individual perforating artery supplies an unexpectedly large area with little overlapping. The proximal perforating artery from the posterior communicating artery supplies the anteromedial thalamus, while the distal perforating artery supplies the posterolateral thalamus as well as the subthalamic area. Lack of overlapping may be due to washing out of the first dye while the second dye is being injected, in the border area where vascular anastomosis is suspected to be present. The dye injection pressure has also to be considered. There is no certain explanation why the areas supplied by two major perforators are not essentially different from those supplied by two perforators after an intervening third one having been occluded. It may be that the stained areas become larger than the actual supplying territories by filling the area which would be supplied by the third perforator, thus becoming comparable in size to those in the cases with two major perforators.

There are usually only very fine perforating arteries originating from the P1 segment of the posterior cerebral artery; therefore, it is technically difficult with the present method to examine territories of those fine perforating arteries less than 0.3 mm, which we did not use for injection.

Infarct models have been made by various authors to demonstrate areas supplied by major arteries or a group of arteries in different animals including man. Focal cerebral ischemia created by electrocoagulation of the lenticulostriate artery in the baboon was recently studied by Yonas et al, which was the first report of an experimental study on an individual perforating artery from the proximal middle cerebral artery. He used silicon rubber to demonstrate the non-infarcted zone. Yoshimoto et al succeeded in making consistent thalamic infarct using multiple clips, apparently resulting in occlusion of a group of perforating arteries. It would be interesting to know whether there is a discrepancy between the area of injection of dye and the area of infarction when the animal is allowed to survive after cauterization of a perforator.

The fact that one perforating artery supplies a relatively large area has clinical implications for the surgery of basilar artery aneurysm or of other lesions in this area, although species difference has to be considered. In fact, we have examined a small number of monkey and bovine brains using the technique presented here, but abundant anastomosing channels in the region of the circle of Willis in these animals made it impossible to obtain constant areas supplied by individual perforating arteries.

References
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