Selective Lenticulostriate Occlusion in the Primate

A Highly Focal Cerebral Ischemia Model

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SUMMARY A highly reliable model for the study of focal cerebral ischemia has been developed using a retro-orbital approach to occlude the lateral lenticulostriate arteries of the baboon. An infarction of the caudate, putamen and the anterior limb of the internal capsule has consistently been produced. Reliability has been attained because the anatomical variations of the lenticulostriate arteries of each animal can be fully appraised, permitting selective vessel occlusion. A well-defined clinical and radiographic lesion has also resulted from this procedure which was clinically well tolerated by all animals. Selective lenticulostriate occlusion provides a new approach to the study of focal cerebral ischemia in the sub-human primate, and serves for the evaluation of proposed therapies for treatment of focal cerebral ischemia.

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AN IMPORTANT GOAL in the study of focal cerebral ischemia is a lower animal model with a lesion that resembles a stroke in man and permits its careful study.1,2 If the model is to be used for the evaluation of a proposed therapy for focal cerebral ischemia, it must be highly reproducible. When subhuman primates are used, an even higher level of reproducibility is necessary to minimize the number of observations and, therefore, the number of valuable animals required to be studied, in order to draw a conclusion of significance. We report a technique for creating a highly reliable focal cerebral ischemic lesion in the baboon that fulfills these requirements as well as providing a unique opportunity to study the primate microcirculation. This technique depends upon selective occlusion of only the penetrating, lateral lenticulostriate (LLS) arteries. This is a fundamentally different approach from previous primate focal ischemia models which have all involved middle cerebral artery occlusion.

Procedure

Nine baboons (Papio cynocephalus/anubis) weighing between 8 and 10 kg were anesthetized with ketamine hydrochloride (Ketalar®) 5.0 mg/kg I.M. and pancuronium bromide (Pavulon®).05 mg/kg. The animals were then intubated and ventilated on a respirator as guided by frequent blood gas determinations. Femoral arterial and venous catheters were inserted for monitoring arterial pressure and for the delivery of normal saline solution.

In order to expose the origins of the LLS arteries without brain retraction, we used a modified retro-orbital approach.4 The animals' heads were secured in a stereotaxic frame and the sagittal plane tilted 10° toward the side of the surgery. After the skin about the right eye and temple was shaved and surgically prepared, a curvilinear incision was made around the lateral half of the orbit just cranial to the zygoma for approximately 1½ inches. The temporalis and masseter muscles were incised and retracted away from the lateral orbital wall. Subperiosteal dissection was then extended into the lateral orbit. To provide additional space for exposure of the apex of the orbit, the posterior chamber of the eye was entered with a 22 gauge needle, and 1.5 to 2.0 ml of vitreous fluid was aspirated. This fluid was saved for later reinjection. A 3 mm self-retaining brain retractor was then positioned to retract the collapsed globe medially. The lateral orbital wall was removed using a small straight rongeur. Bone removal of the greater sphenoid wing was continued until the dura was exposed. The inferior lateral wall of the orbit was then removed with a 3 mm angled Kerrison rongeur. A one cm high craniectomy was then created with the Sylvian fissure being approximately one-third of the way from its base. The fissure was identified by a slight thickening of the dura and by a line of increased adherence to the bone. Bone removal was extended medially to the rim of the optic canal around which the bone thickened markedly. The dura was then incised and folded inferiorly, exposing the arachnoid over the middle cerebral artery.

In this region the middle cerebral artery tranverses the brain surface, arising medially behind the optic canal and coursing laterally to enter the Sylvian fissure. After microdissection of the arachnoid on the superior aspect of the middle cerebral artery, the orbito-frontal artery and its relationship to the LLS arteries could be evaluated (fig. 1).
The LLS arteries were coagulated using a low current setting with fine tipped insulated bipolar forceps. In 3 animals the initial segment of the orbito-frontal artery, from which the LLS arteries often arise, also had to be coagulated in order to assure proximal occlusion of the LLS vessels. The dura was reapproximated with 8-0 nylon sutures, and the globe was reexpanded by injecting the vitreous fluid into the eye. Following reexpansion, the globe covered the retro-orbital cranietomy site. The musculature was then closed in layers, and the skin was reapproximated.

The second and sixth animals had “sham” procedures with exposure but without coagulation of the LLS arteries. The sixth animal served as his own control, and one month later his LLS arteries were occluded on the opposite side.

All animals received lincomycin hydrochloride (Lincocin®) 10 mg/kg and streptomycin sulfate® 10 mg/kg each for 5 days starting the morning of surgery. On awakening, the animals were returned to their cages and monitored daily, thereafter, for neurological alterations. All of the animals had CT scans during the second or third week following vessel occlusion. Femoral cerebral angiography was performed on the first 4 animals.

Approximately 3 weeks following the procedure and within 24 hours after the CT scan, the animals were killed and their brains formalin fixed as follows: While deeply anesthetized with sodium thiopental (Pentothal® 30 mg/kg), each animal had its abdominal aorta and inferior vena cava cannulated. The saline perfusion was followed by 1000 ml of 10% buffered formalin solution. The brains were then removed from the skulls and placed in 10% buffered formalin solution for one week. They were studied for external anatomical detail under 15 and 25 power magnification and photographed. The brains were cut into 4 mm slices for determination of size and location of infarctions. Xeroradiography§§ of the silicone injected sections was employed to obtain plainer images of the vascular system at each level.

Results

All 9 animals returned to full alertness within a few hours after the operation. There were 10 operations since the sixth animal had undergone both a sham and an experimental procedure. All eight animals with LLS arteries surgically occluded had weakness on the contralateral half of the body. These animals were consistently weakest in the upper extremity with varying degrees of facial and leg weakness. None of the animals was able to grasp or use the fingers of its left forelimb for climbing, although all retained gross proximal tone and movement of the limb. While 2 of the animals had no apparent left leg weakness, 2 were totally unable to grasp the cage bars with either the left fore or hind limbs. The remaining 4 animals were able to move the hind limb, but appeared, at times, to have difficulty grasping cage bars. Although there were improvements in these findings over the 3 weeks following surgery, the forelimb hand weakness remained complete in all animals. The 2 “sham”-operated animals that had only exposure and manipulation of the LLS arteries recovered without apparent neurological deficit.

The animals, in general, appeared to adjust rapidly to their deficits and climbed about their cages on the first postoperative day using the uninvolved limbs. No basic alterations of behavior were apparent within a few days after the procedure, e.g., aggressive animals retained this characteristic. All wounds healed well, and all animals had normal appearing eyes and eye movements just prior to sacrifice, 3 weeks after the lesion was created. The animal that had a sham procedure on the left side and an occlusive procedure on the right retained some vision with awareness of movement objects about him in spite of the fact that both eyes had undergone vitreous decompression.

In all of the 4 initial animals that had angiography both middle cerebral arteries were patent and LLS occluded. The 4 animals that were injected with radiopaque silicone rubber, but did not have angiography, also were found to have constant LLS occlusion with patency of the remainder of the vasculature (fig. 2).

The CT scans, which were performed during the third week in each animal, showed well-defined areas of low density and within 24 hours after the operation. There were 10 operations since the sixth animal had undergone both a sham and an experimental procedure. All eight animals with LLS arteries surgically occluded had weakness on the contralateral half of the body. These animals were consistently weakest in the upper extremity with varying degrees of facial and leg weakness. None of the animals was able to grasp or use the fingers of its left forelimb for climbing, although all retained gross proximal tone and movement of the limb. While 3 of the animals had no apparent left leg weakness, 2 were totally unable to grasp the cage bars with either the left fore or hind limbs. The remaining 4 animals were able to move the hind limb, but appeared, at times, to have difficulty grasping cage bars. Although there were improvements in these findings over the 3 weeks following surgery, the forelimb hand weakness remained complete in all animals. The 2 “sham”-operated animals that had only exposure and manipulation of the LLS arteries recovered without apparent neurological deficit.

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density within the corpus striatum on the side of vessel occlusion (fig. 3).

At autopsy, all wounds were found to be well healed, and minimal scarring was found about the craniectomy site and the middle cerebral artery. Under binocular magnification selective occlusion of the LLS was verified in all animals in which the LLS arteries were intentionally occluded at surgery. In one silicone-injected animal in which the proximal orbito-frontal artery was coagulated along with the LLS arteries that arose directly from it, the distal part of this vessel was seen to be patent due to leptomeningeal collaterals.

Gross pathological study of the brain sections revealed well defined cerebral infarctions in all animals in which the LLS arteries were intentionally occluded. An area of gross tissue softening and early cystic formation consistently involved the most lateral part of the globus pallidus, the more superior and posterior part of the head, the body of the caudate nucleus, nearly all of the putamen, and the intervening anterior limb of the internal capsule (fig. 4). The area of necrosis consistently included the distal body of the caudate and the adjacent centrum semiovale. The brains of animals in which the LLS arteries were manipulated but not occluded showed no evidence of cerebral infarction.

Anatomical Considerations

The blood supply to the corpus striatum (caudate nucleus, putamen, and globus pallidus) is primarily derived from the medial and lateral lenticulostriate arteries with a variable contribution to the medial globus pallidus from the anterior choroidal artery. While the medial lenticulostriate arteries supply blood to the most anterior and caudal striatum, the LLS arteries provide the primary blood supply to its larger and more posterior lateral portion.

The LLS arteries of the baboon have been reported to arise from the lateral portion of the middle cerebral artery prior to its bifurcation and either directly from or near to the orbito-frontal artery. The orbito-frontal artery is a prominent cortical vessel in the baboon and is often found within a few mm of the middle cerebral artery bifurcation. Watanabe and Laurent et al. reported that the LLS arteries of the baboon most often arise as a cluster of vessels directly from the orbito-frontal artery. We have observed this occurrence in 6 of the 14 examined middle cerebral arteries (10 surgical observations and 4 postmortem following the injection of silicone rubber). We have also found, however, that the relationship of the LLS arteries with the orbito-frontal artery may vary significantly (fig. 5). In 6 specimens the LLS arteries arose from both the orbito-frontal and the middle cerebral arteries. In the remaining 2 specimens the LLS arteries arose independently of the orbito-frontal artery. This latter arrangement was observed when the orbito-frontal artery arose from the middle cerebral artery either most proximally or most distally.

The LLS arteries of the baboon either course medially (10 of 14 specimens) along the middle

**Figure 2.** A xeroradiograph of the 4 mm thick coronal brain section following silicone injection of the vasculature. Note the absence of LLS arteries arising from the animal's right middle cerebral artery.

**Figure 3.** Representative coronal computerized tomographic scan through the level of the corpus striatum performed three weeks following LLS occlusion.

**Figure 4.** Axial tissue section through caudate nucleus (Caud.), putamen (Put) and anterior limb of the internal capsule (IC). Arrows mark limit of infarction. Tissues perfused by the posterior circulation are not filled with dark silicone rubber.
FIGURE 5. Variations of the origins of the LLS arteries about the middle cerebral artery (MC). Most commonly these vessels arise from (left) or adjacent to the orbital frontal artery (OF) (center). A separate origin of the LLS from the OF was also encountered (right).

within the substance of the brain, the LLS arteries have been observed to initially sweep medially and then laterally as they "fan out" in an anterior-posterior direction within the body of the putamen. They then provide the primary blood supply to this structure as well as to portions of the head and body of the caudate nucleus, the lateral globus pallidus, and the anterior limb of the internal capsule. Distally, they provide blood to the more cephalad and posterior caudate nucleus internal capsule and the centrum semiovale. Although Wanabe7 suggested that the LLS arteries may also supply blood to the claustrum of the baboon, this area was not included in the infarction in any of our specimens.

The major factor in determining whether an infarction will follow the occlusion of a vessel is the degree of collateral circulation that can develop distal to the point of occlusion before an irreversible ischemic insult occurs. The major routes for collateral blood flow distal to the circle of Willis in the baboon and man are through leptomeningeal anastomoses with adjacent cortical vessels. These collateral channels are undoubtedly responsible for the repeated observation of significant blood flow distal to a focal occlusion of the middle cerebral artery and for the variability in the size of the resulting infarction.9–18

A second major collateral route that is functional in many lower animals and some non-human primates is an extensive system of anastomoses between the medial and lateral lenticulostriate vessels of the baboons, anastomoses at this level were not observed in any of our injected specimens examined at 25 power magnification.

Discussion

In 1847, Flourens first attempted to create an experimental model of focal cerebral ischemia by injecting various embolic materials into the cervical carotid artery of dogs. A more direct approach was taken by Peterson et al. in 1937 when they reported a subfrontal surgical procedure for occlusion of the middle cerebral artery in the dog. Since then, most models for focal ischemia have involved the middle cerebral artery because of its accessibility by direct surgical approaches as well as by flow-directed embolic techniques.9–14

The direct surgical approaches for occlusion of the middle cerebral artery remained essentially unchanged until 1966 when Sundt and Waltz introduced a retro-orbital approach in order to minimize tissue damage due to brain retraction. An even more direct, transorbital approach was introduced by Hudgkins and Garcia in 1970. These investigators reported that the transorbital route was technically less demanding and had the advantage of avoiding any brain retraction, while still providing direct visualization of...
the internal carotid and middle cerebral artery junction. Although both techniques for proximal middle cerebral occlusion have provided useful models for study of various aspects of focal ischemia, neither technique produces a cerebral infarction consistent in extent or location.7-14, 16-18 They produced minimal to massive cerebral infarctions in primates in a way not under control of the investigator. It is probable that the extent of infarction is dependent on the quality of pre-existing collateral circulation, as well as the degree to which any of the lenticulostrate arteries might have been compromised during clip placement. Ten to twenty percent of these animals were also lost to chronic studies because of massive and rapidly fatal cerebral infarctions.11-18 Undefined surgical artifacts may also result from entering the cranium and from brain retraction.29, 30 Distal middle cerebral artery autoregulation may also be lost following damage to the vasonervorum when the middle cerebral artery is crushed in an occluding device.31

Embolic techniques for the creation of focal cerebral ischemia have the advantage of closely approximating the mechanism of clinical intravascular occlusion due to emboli, as well as of avoiding surgical entry into the skull.7, 8, 28 Molinari described the injection of a 7.0 mm long silastic cylinder into the cervical carotid artery of the dog and the monkey (Macaca mulatta). This often caused occlusion of the initial segment of the middle cerebral artery.7, 28 The model produced deep cortical infarctions more predictably than the single point occlusion techniques.89 The increased reliability for lesion formation is most likely due to the fact that with the cylinder lying within the middle cerebral artery, retrograde collateral blood flow is blocked as are the origins of many of the LLS arteries. This model has served as an effective means of studying cerebral reperfusion90, 94 and various therapeutic agents for the treatment of focal ischemia in the dog95, 96 and primate.97 This technique is limited because the exact area of occlusion within the middle cerebral artery is variable as are the anatomical configurations of the LLS arteries. Thus, a degree of variation in lesion size and location is inevitable with embolic methods. Procedures using emboli have also resulted in the loss of 10 to 20 percent of the animals due to a rapidly fatal massive cerebral edema that has followed occlusion of the initial few mm of the middle cerebral artery.98-101 In our experience with baboons, an additional 40% of these valuable animals have been lost from our study due to inability to direct the embolus accurately into the middle cerebral artery.

We have found that the highly selective focal cerebral infarction that is created by occluding only the LLS arteries provides a reliable means of studying a lesion which is definable clinically, radiographically, and pathologically.

As with cerebral artery occlusion the clinical deficits i.e., arm and a variable degree of leg weakness, occurred in each animal. However, unlike middle cerebral artery occlusion where a larger cortical area is made ischemic, our animals have rapidly adjusted to these deficits and resumed their normal behavior patterns.102

In every animal, an area of low density has consistently been seen on CT scan and has been of adequate size to permit in vivo study of stroke evolution (see fig. 3). The limited area of consistent subcortical infarction that is produced by LLS occlusion should provide a useful model to evaluate therapeutic regimens which on initial studies in lower animals have been encouraging enough to warrant evaluation in a subhuman primate.

As with other surgical procedures for the production of focal cerebral ischemia, this model can be criticized because it requires a craniectomy and manipulation of cerebral vessels. We have not observed significant cortical tissue alterations at the operative site. The major areas of infarction occurred distal to the site of occlusion but within the distribution of the LLS arteries. The practice of approximating the dura and of maintaining the normal orbital structures has also resulted in minimal scarring about the middle cerebral artery. In the 2 animals in which vessels were exposed and manipulated but not occluded, we did not observe clinical deficits or gross pathological lesions.

The use of electrical coagulation to accomplish vessel occlusion can also be criticized because of the possibility of tissue damage caused by current or heat injury to adjacent tissues. The use of a fine bipolar forceps at a very low power setting, however, causes...
only a focal lesion limited to the tissues between the tips of the instrument. A possible problem of this procedure is that it requires microsurgical technical competence. We believe, however, that this level of expertise is no greater than that demanded for any other microsurgical procedure routinely performed today and that it is not beyond the grasp of most competent animal laboratory personnel.

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