Segmental Middle Cerebral Artery Occlusion in Primates: An Experimental Method Requiring Minimal Surgery and Anesthesia

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Abstract:
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- Due to certain anthropomorphic features of the cerebral circulation, monkeys are generally preferable to lower species in experimental models of stroke. By injecting specially molded silicone cylinders through internal carotid artery cannulas, segmental occlusions of the middle cerebral artery were produced in macaques. This embolic method produced cerebral infarction in all instances while the integrity of skull and intracranial collateral circulation were preserved. Because only neck surgery was required, local anesthesia could be used, permitting observation of acute infarctions in sedated, conscious animals.

Additional Key Words embolic method primate stroke model local anesthesia

Introduction
- Both direct ligation of blood vessels and intra-arterial injection of foreign substances have provided the means for studying experimental cerebrovascular lesions for well over a century.

Over the years, direct extrinsic ligation of cerebral vessels using spring clips has been used extensively and effectively in dogs, cats, and primates, paralleling the development of improved optical equipment and surgical techniques. While application of a metal clip offers the desirable features of direct visual control and reversibility after known time intervals, a prolonged period of anesthesia is required at the outset because of the prerequisite craniotomy or orbital surgery. Moreover, single clips occlude vessels at points no more than 1 or 2 mm in width and allow perfusion of distal branches proportionate to the quality and quantity of collateral circulation.

Emboli with plastic materials have been used successfully in dogs to occlude longer cerebrovascular segments, but segmental occlusion in primates usually has been accomplished by application of multiple clips.

This report describes an embolic method originally developed in dogs, adapted to the anatomical variables encountered in the primate species, Macaca mulatta. The technique permits segmental middle cerebral artery occlusion after minor extracranial surgery and under minimal anesthesia.

Methods
- Small amounts of the silicone rubber compound, Microfil, were mixed in sterile containers using the manufacturer’s recommended formula. Avoiding air bubbles and bacterial contamination, the liquid mixture was gently drawn by vacuum suction into polyethylene tubes of selected internal diameter (Intramedic®, PE 200, PE 205). Filled tubes were sealed with surgical clamps and set aside for 24 hours. Yellow Microfil® (MU 122)* was used most often, while the orange-colored (MU 117)* material was preferred when repeat experiments were performed upon a single animal. The lead contained in both pigments allowed visualization of the polymer on x-ray films.

Emboli molded in Intramedic tubing, PE 200, had a diameter of 1.41 mm, while those cast in PE 205 measured 1.58 (1.6) mm. Segments were cut from the tubing containing continuous filaments of the polymerized silicone rubber compound, and cylindrical emboli were extruded from the cut segments by simple digital compression. Free segments of the elastic polymer were inserted into the connecting tips of disposable syringes containing 3 to 5 cc of sterile physiological saline and cut to selected lengths ranging from 4 to 8 mm.

Healthy adolescent monkeys (Macaca mulatta) of either sex, weighing 2.8 to 4.7 kg, were premedicated with phencyclidine (0.5 mg per kilogram) and atropine (0.1 mg per kilogram). Initially animals were given a repository dose of penicillin in oil, 300,000 U, I.M. after surgery, but, later, •
another 300,000 U of aqueous penicillin was added to the premedication schedule.

Twenty-two common carotid artery cutdowns were performed in 18 animals. Twelve of these operations were done under general anesthesia using intravenous pentobarbital, 4 mg per kilogram. Ten animals received only local skin infiltration with 2 ml of 1% procaine at the surgical site.

Sham operations were performed on two monkeys in the group receiving general anesthesia; the common and internal carotid arteries were tied in the neck, but emboli were not injected.

Twenty embolisms were produced in the remaining 16 animals using the following injection techniques: Medicut® cannulas (No. 16 or No. 18 gauge) were inserted into the internal carotid artery through longitudinal common carotid arteriotomies; the arterial incisions were made between two temporary ligatures just proximal to the carotid bulb. Bleeding was controlled by a stopper in the cannula and tension on two untied ligatures. Single cylinders of elastic, pigmented, radiopaque, silicone rubber of preselected diameter and length were then injected through the internal carotid artery cannula and flushed into the arterial system.

Since diameters of 1.4 to 1.6 mm exceeded the bores of No. 18 and No. 16 gauge cannulas, respectively, emboli passed through the cannulas by stretching to greater length but smaller diameter. Due to the inherent plasticity and elasticity of the material, emboli returned toward preinjection diameters and lengths as permitted by the diameter of the surrounding conduit. Therefore, gentle forward pressure was necessary to propell the emboli through the cannulas. After minimal experience, a palpable pressure drop signaled clearance of the embolus from the cannula into the internal carotid artery. Backflow did not appear, however, until the embolus was advanced to a point distal to the sources of collateral circulation to the internal carotid artery. Thus, after injection, emboli were advanced by intermittent syringe pressure to allow both palpation of the pressure drop and observation of the first appearance of backflow. The latter event marked the intracranial arrival of the embolus, since retrograde carotid flow in the monkey could originate only from the ophthalmic artery or the circle of Willis.

Arteriotomy sites were closed using 9-0 microsurgical suture in seven operations; proximal and distal common carotid ligatures were tied to isolate the incised segment in all other cases.

Animals were killed after one hour to 30 days of clinical observation. Brains were usually perfused, in situ, with 10% formalin solution and then immersed in formalin for one to two weeks. Fixed brains were studied for correlation of site and extent of vascular occlusion with distribution and quality of cerebral lesions. Representative sections were made from blocks of infarcted tissue and selected control areas.

Results

Carotid injection of elastic cylinders produced segmental occlusions in the middle cerebral artery distribution in all cases. Seventeen occlusions (80%) involved the horizontal segment of the middle cerebral artery proximal to the candelabra formation, the M-1 segment as classified by Fischer. In three experiments, multifocal segmental occlusion of the pericallosal and middle cerebral branches were caused by fractionation of the embolus during or after injection.

The two sham neck operations produced no clinical or pathological evidence of cerebral infarction or ischemia.

The diameter of the proximal segment of the middle cerebral artery (MCA) in fixed brain specimens ranged from 0.7 mm to 1.1 mm in this series of macaques. In a previous report using this method, emboli of 1.6 mm diameter were found to occlude the 1 mm diameter segment of MCA in dogs. Therefore, in order to selectively occlude the main stem of the MCA near its origin from the anastomotic circle of Willis, a ratio of approximately 1.5:1 was applied in selecting Intramedic®, tubing sizes PE 200 (internal diameter 1.4 mm) and PE 205 (internal diameter 1.6 mm) for molding emboli for use in monkeys.

Slight variations in diameter of the MCA were independent of body weight in animals of this size and age. The internal carotid arteries in the neck, however, were more variable and were weight-dependent. The internal carotid easily admitted a No. 18 gauge cannula in animals under 3.5 kg, and a No. 16 gauge could be used in larger monkeys. Emboli molded in PE 200 tubing (1.4 mm diameter) passed through the lumina of the No. 18 gauge cannulas with minimal resistance. Similarly, emboli of 1.6 mm diameter (PE 205) easily passed through No. 16 gauge cannulas. Either size was satisfactory for occlusion of the proximal MCA segment. The choice between sizes was determined by the gauge of the largest cannula accommodated by the surgically exposed internal carotid artery.

The final placement of emboli within the cerebral arterial tree was dependent upon the size and quality of the embolic particle injected and, to a lesser extent, upon anatomical variables in the branching of the middle cerebral artery.

Figure 1 is an x-ray film showing an embolus in the proximal middle cerebral artery segment just lateral to the optic foramen. The film was made without special preparation, using ordinary roentgenographical equipment. Projection was anterior to posterior in the long axis of the right orbit. The embolus was 7 mm × 1.5 mm at injection.

The distribution of infarctions varied with the length of the embolus injected. Emboli measuring 7 mm or more before injection occluded the entire horizontal segment from the anastomotic circle to the branching at the candelabra formation in the Sylvian fissure. Figure 2 shows the same embolus demonstrated radiographically in figure 1 in the brain specimen. The embolism extended from a point proximal to the origin of the posterior communicating artery through the MCA to the origin of the large orbitofrontal branch. Longer emboli of this type obstructed the origins of all the penetrating branches.
Plain A-P roentgenogram made in the long axis of the right orbit 24 hours after embolism. The arrow denotes the curvilinear positive shadow of an experimental embolus in the proximal MCA. Note the relationship of the embolus in MCA to the negative shadow of the optic foramen medially.

of MCA including the anterior choroidal artery, proximally.

Distally, the orbitofrontal artery was usually the first branch of the MCA causing an abrupt reduction in the caliber of the parent vessel in the Sylvian fissure. However, in some hemispheres, that branch arose medial to the fissure as a small vessel from the anterior wall of the M-I segment. In such instances the first major branch contributing to the candelabrum was the anterior temporal. The distal extent of the occlusion was slightly more lateral in hemispheres with the latter vascular pattern; the smaller orbitofrontal branch was then occluded at its origin in the same manner as the penetrating vessels.

Figure 3 is a coronal section of the brain shown in figure 2. Twenty-four hours after embolism, the brain shows pallor, loss of vascular markings and slight swelling throughout the territory supplied by the surface and penetrating branches of MCA. The caudate, pallidum, putamen, claustrum, internal and external capsules are all involved. Shorter emboli of approximately 4 to 5 mm occluded the lateral portion of the horizontal segment, but the first few millimeters of the MCA remained patent. Therefore, deep structures supplied by the anterior choroidal artery, the first penetrating branch of MCA, were spared.

Outdated Microfil (shelf-life four months) and material molded more than two days before use produced friable emboli of generally poor quality. In three of our experiments (20%), segmental occlusions involved distal MCA branches in the Sylvian fissure and the pericallosal artery. Patchy, bland areas of cortical infarction were produced distal to each occluded segment. Deep hemispherical structures were spared, however, unless occlusions also involved the horizontal MCA segment proximal to the candelabra formation. Multifocal occlusions did not occur when freshly prepared emboli were used.

In general the severity of focal residual signs varied directly with the completeness of horizontal segment occlusion. Occlusions of the entire segment from the origin at the carotid trifurcation to the branching at the candelabra caused severe contralateral sensory, motor and visual deficits; shorter occlusions, sparing the proximal MCA, caused less severe impairments. Occlusions of distal cortical branches of MCA and the pericallosal artery occlusion produced only transient signs and negligible residual impairment.

Focal neurological signs such as hemiplegia and tonic eye deviation were observed immediately after embolism in animals given only phencyclidine and local procaine, while six hours or more were required to evaluate the effects of embolism when barbiturate anesthesia was used.

Some lesions obtained from animals used in chronic experiments involving more than four days' survival showed histological evidence of bacterial contamination at the periphery. Lesions from animals meticulously treated with antibiotics before and after embolism were free of biological artifacts.

Detailed clinicopathological correlation will be the subject of a subsequent report.

Discussion

In applying this previously reported model to primates, a number of species-dependent, anatomical variables were encountered. In the dog the major extracranial anastomosis between the maxillary and internal carotid arteries and the large, well-developed posterior communicating arteries differs markedly from primate vascular patterns. Intracranially, the branching of the M-I segment of MCA into the so-called "candelabra" occurs on the hemispherical surface of canine brains, while in the anatomically more complex simian brain, this arborization occurs deep in the Sylvian fissure on the insular cortex. The relative proportions of the middle cerebral to posterior communicating arteries differs markedly from primate vascular patterns. Intracranially, the branching of the M-I segment of MCA into the so-called "candelabra" occurs on the hemispherical surface of canine brains, while in the anatomically more complex simian brain, this arborization occurs deep in the Sylvian fissure on the insular cortex. The relative proportions of the middle cerebral to posterior communicating arteries of the macaque may be seen in figure 2. These anatomical features make the primate pattern the more credible model of the human cerebral circulation.

Despite the relative constancy of patterns of the cerebral vasculature among primate species including man, the anthropomorphism of the circle of Willis is far from perfect even in the macaque, particularly...
Experimental embolus in the right MCA of specimen taken from the same animal shown in figure 1. The embolus measured 7 mm X 1.5 mm when injected. The occlusion extends from the internal carotid a., proximal to the posterior communicating a., and is impacted at the origin of the orbitofrontal a. distally. Emboli of this length occlude the origins of all of the major penetrating branches of MCA, including the anterior choroidal.

Gross cross section of specimen shown in figure 2 showing distribution of ischemia. The affected side is pale with a paucity of vascular markings in both the superficial and deep territories supplied by the MCA. Note the degree of brain swelling indicated by the ventricular asymmetry. This specimen was obtained 25 hours after experimental embolism.

Because the configuration of the candelabra was somewhat variable in this species, the distribution of infarctions varied slightly with the vascular anatomy. This was considered acceptable since similar variables occur in human pathology and because precise clinicopathological correlations can be made using our model. Furthermore, horizontal segment occlusions always caused deep infarctions in the distribution of
the lateral lenticulostriate arteries despite variations in the angioanatomy and length of embolus. The use of longer emboli, approximately 7 mm in length, compensated for variations in the distal patterns of arborization and assured obstruction of the more medial penetrating branches as well.

The diameters of emboli optimal for occlusion of the horizontal segment of the MCA were approximately one and one-half times the diameters of the formalin-fixed arterial segments. Although this ratio was determined empirically, further precision based upon measurements of fixed arterial specimens would not be fruitful because of the inconstant shrinkage artifact. Moreover, these segments are reactive in vivo, and the diameters vary with pathophysiological changes. Using this ratio, the inherent elasticity of the silicone cylinders maintains centrifugal pressure upon the intimal surface of occluded segments despite a considerable range of reactive vasodilatation.

This method produced segmental occlusion of the MCA while leaving the meningoencephalic collateral circulation anatomically and physiologically intact. Meyer10-11 has discussed the need to apply multiple ligatures to isolate a vascular segment from collateral flow. In studies comparing the effects of temporary ligation to permanent ligation of cerebral arteries, the model used for permanent ligation has usually been surgical division of the artery between two clips.15,16 While such occlusions are permanent indeed, they are also segmental. The severity and distribution of the cerebral lesions might well be different from those caused by nonsegmental occlusion with single removable clips. Moreover, surgical division may cause autonomic neurotomy, with denervation of the pial vessels distal to the occlusion. Vasospasm caused by extrinsic compression of cerebral vessels also may introduce physiological if not morphological artifacts in experimental models of infarction and ischemia.

Because our only surgical defect is in the neck, the brain specimen is free from the pathological artifacts produced by traumatic manipulation and electrocautery.17 The entire cranium remains intact and suitable for acute or chronic electrophysiological monitoring. Physiological data recorded in vivo may be collated with precise localization of the vascular occlusion by x-ray or postmortem examination. The abbreviated preparation time and limitation of surgery to soft tissues in the neck eliminate the need for prolonged anesthesia and therefore the specific effects of barbiturate drugs are removed from morphological and pathophysiological studies.

Using microsurgical techniques, the internal carotid arteriotomy may be closed, leaving the lumen patent for cranial blood flow after embolism.

This embolic technique is subject to certain technical and biological artifacts which can be avoided or controlled. These include the accidental fragmentation of embolic cylinders during injection and secondary fragmentation of cerebral lesions in chronic experiments.18 Both may be controlled by using fresh materials, meticulous sterile technique and generous antibiotic treatment before and after surgery.

Because of minor variations in the branching of individual middle cerebral arteries, the distal limit of the occluded segment may vary by a few millimeters. These anatomical variables may be compensated for by using longer emboli (7 mm) of the largest diameter admissible through an internal carotid artery cannula.

Conclusion

In this series infarctions of the putamen and internal capsule were constant findings in horizontal segment occlusion regardless of length of embolus used or configuration of the candela. The overall rate of horizontal segment occlusion (80%) improved with experience and approached 100% as technical artifacts were identified and corrected. The method described offers a reasonable alternative to craniectomy and general anesthesia in the laboratory investigation of cerebral infarction. Intrinsic segmental occlusion of the middle cerebral artery may be preferable to extrinsic ligation in certain pathophysiological experiments.

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