Experimental Cerebral Infarction. I. Selective Segmental Occlusion of Intracranial Arteries in the Dog

BY GAETANO F. MOLINARI, M.D.

Abstract: Experimental Cerebral Infarction. I. Selective Segmental Occlusion of Intracranial Arteries in the Dog

1. Cylinders of silicone rubber can be molded to any desired diameter and cut to length, yielding a highly pigmented, radio-opaque, elastic bolus suitable for intravascular injection and embolization.
2. Emboli of this type can be localized by x-ray in vivo and at post mortem for clinicopathological correlation studies.
3. Segments of specific vessels of known diameter can be selectively occluded with a high degree of predictability.
4. The caliber of the proximal segment of the middle cerebral artery is remarkably constant in dogs. The extracranial arteries, however, vary proportionately to the body weight of the animal.
5. Proximal segmental occlusion of the middle cerebral artery results in occlusion of the origins of the lenticulostriate vessels. This produces a deep ischemic infarction of basal ganglia and internal capsule. The cerebral cortex is spared due to the integrity of meningocerebral anastomotic network.

ADDITIONAL KEY WORDS: middle cerebral artery, silicone rubber, cerebral embolus, carotid artery

Introduction

Experimental cerebral infarction has been produced in animals by the introduction of a wide variety of foreign bodies into the internal carotid system.

The optimal material for experimental emboli should be radio-opaque and easily identified in pathological specimens, and should produce cerebral lesions which resemble spontaneously occurring clinical infarctions. Standardized doses of embolic material should cause reproducible vascular occlusions and predictable cerebral lesions.

The substances used in earlier studies seldom had more than one of these distinctive characteristics. Because the size of the experimental embolus is limited by the diameter of needles of cannulae used in delivery to the internal carotid system, most embolic experiments have dealt with the meningocerebral and microvascular circulations.

Although homologous blood clots predictably cause gross hemorrhagic infarction, a relatively large volume (0.2 cc) is required, compared to the intravascular volume of principal cerebral arteries (4.5 mm³ for the first segment of the middle cerebral artery). In addition, injected clots can seldom be identified in postmortem specimens because of fractionation into small particles or breakdown by fibrinolytic activity.

Liquid vinyl acetate (0.2 cc or 0.3 cc), introduced into an internal carotid artery, promptly hardens into a continuous filament occluding the internal carotid, middle cerebral, and sometimes other intracerebral arteries. Lesions produced by this method are less often hemorrhagic, but long filamentous occlusions seldom occur in clinical pathology and, furthermore, prevent functioning of the collateral circulation.

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EXPERIMENTAL CEREBRAL INFARCTION I

This study will describe a method for segmental occlusion of intracranial arteries in the dog which permits selective infarction of either deep or superficial structures.

Materials
Microfil* is a commercially available silicone rubber compound which is vividly pigmented and polymerizes at room temperature within two hours when mixed with solute and catalyst. The compound has been used extensively in anatomical research on the microcirculation of many organ systems. When it is injected in liquid phase into the blood vessels of dead animals, polymerization results in an accurately detailed cast of the vascular bed perfused. Two of the available colors, orange and yellow, contain a lead-chrome matrix which offers a high x-ray absorption.

Method
Yellow or orange Microfil solute was added to equal amounts of solvent (by weight) and catalyst added. The mixture was drawn up in liquid phase into polyethylene tubing of several diameters. The ends of the filled tubes were sealed with wax and the system was allowed to polymerize for 24 hours. Prior lubrication of the interior of the tubing with glycerine prevented adherence of the polymerized material. Catheters admitting 15-gauge, 18-gauge, and 20-gauge needles yielded embolic cylinders measuring 1.6 mm, 1 mm, and 0.8 mm in diameter respectively. In each experiment, an embolus of selected diameter was prepared by cutting the tubing containing the rubber compound to the desired length. The resulting rubber cylinder was then extruded en bloc by simple pinch pressure at one end of the casing. Each cylinder was then inserted into the hub of a 10 ml syringe filled with normal saline (fig. 1A and B).

Carotid artery dissection was performed on 25 healthy mongrel dogs under pentobarbital anesthesia (5 mgm/kg body weight). Despite the range of weight from 9.8 kg to 28 kg, there was little variation in the size of the brain specimens and in the diameters of intracranial arteries. While there was more variability in the size of the internal carotid artery in the neck, the extracranial vasculature was sufficiently elastic to allow insertion of a 16-gauge or 17-gauge Rochester needle in all cases.

Cylinders measuring 5 mm by 1.66 mm† were injected into 14 dogs. In another six experiments, emboli were given which measured 5 mm by 1 mm. Smaller emboli were prepared by...
cutting slender filaments (50 mm by 0.86 mm) into 1 mm particles. Showers of these small emboli were used in five experiments.

Intra-arterial pressure was not measured during the injections into the extracranial vessels. Direct cannulation of the internal carotid artery produced the pressure and blood flow gradients illustrated in figure 2. That is, with the internal carotid artery obstructed by the cannula which was held in place by a ligature, pressure in the dead space distal to the ligature and proximal to the anastomosis could be maintained at the systemic blood pressure level by communication with the unobstructed external carotid through the maxillocarotid anastomotic branches. Blood can flow in this system, however, only in the direction indicated by the arrows in figure 2. Emboli, contrast media, isotopes, etc., introduced into this "dead space" need to be flushed in with physiological saline solution under pressure generated in the syringe. So long as that exogenous pressure does not exceed systemic arterial pressure, the embolic cylinder or contrast medium cannot flow extracranially through the anastomotic artery (counter current to blood flow). Once the embolus is delivered to a position in the internal carotid distal to the anastomosis, exogenous pressure is no longer required, "back flow" appears in the syringe and the embolus is carried to its final site of occlusion by endogenous pressure and flow gradients.

Although 1.66 mm is somewhat larger than the internal diameter of a 16-gauge polyethylene needle, the silicone rubber polymer passes through by elongation due to its inherent elasticity (fig. 1C). The elongated bolus could be seen entering the intravascular portion of the transparent needle; release of the embolus into the blood stream was signaled by the sudden fall in resistance to pressure on the plunger. Three to five milliliters of saline were required to wash in the emboli, using intermittent pressure on the syringe. The appearance of "back flow" indicated the delivery of the bolus beyond the maxillocarotid anastomosis and progression into the intracranial vasculature. Distal ligation of the internal carotid artery minimized bleeding after withdrawal of the cannula, while perfusion of the ipsilateral cerebral

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*Molded in Intramedic PE 90/S36 Polyethylene Tubing.*

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*Figure 2*

Schematic illustration of the direction of blood flow in external carotid-maxillocarotid anastomotic system when a cannula is placed in the internal carotid artery. The appearance of "back flow" in the cannula signals progression of the embolus beyond the site of anastomosis.
hemisphere was uninterrupted due to the anastomosis between the internal carotid and maxillary arteries.\textsuperscript{11}

In chronic experiments the neck incisions were closed with clips and the animals allowed to recover from anesthesia. Intramuscular antibiotics were given to surviving animals daily for up to seven days. Infection was not a significant complication despite the fact that surgery was "clean" but not sterile.

Eight animals were sacrificed at the conclusion of acute experiments. Seventeen dogs were maintained for 24 hours to 18 days, to permit clinical observations and to allow chronic pathological changes to develop. At the time of sacrifice, the brains were perfused in situ with 500 ml of 10\% formalin to facilitate postmortem dissection and removal.

The diameter of the first segment of the middle cerebral artery distal to the circle of Willis was measured on the unaffected side in each specimen. Direct measurements were performed using a millimeter rule under a dissecting microscope. Variations in diameters of individual arteries were very small. To provide a larger surface for comparison of variations, middle cerebral arteries were split longitudinally and opened, and the breadth of the internal surface was measured. This measurement was the internal circumference of the vessel; from it the diameter was calculated. Gross specimens were cut into serial coronal sections approximately 5 mm thick; histological sections were prepared from whole coronal planes to allow comparison between affected and control hemispheres. Hematoxylin and eosin stains were made of selected representative sections.

**Results**

Intracerebral vascular occlusions occurred in all specimens. Correlations between embolic size and site of occlusion are summarized in table 1.

Gross sections of the proximal segment of the middle cerebral arteries consistently mea-

<table>
<thead>
<tr>
<th>Exp. #</th>
<th>Body weight, kg</th>
<th>Diameter of proximal MCA, mm</th>
<th>Diameter of embolus injected mm</th>
<th>Site of vascular occlusion</th>
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<tr>
<td>1</td>
<td>12.0</td>
<td>1.1</td>
<td>1.00</td>
<td>Proximal MCA*</td>
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<tr>
<td>2</td>
<td>10.0</td>
<td>0.9</td>
<td>1.00</td>
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<td>1.00</td>
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<td>8</td>
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<td>1.1</td>
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</tr>
<tr>
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<td>0.9</td>
<td>1.66</td>
<td>Proximal MCA</td>
</tr>
<tr>
<td>21</td>
<td>18.0</td>
<td>1.1</td>
<td>Each</td>
<td>Multiple meningocerebral blood vessels, mainly in MCA distribution but with occasional particles</td>
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<td>0.9</td>
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<td>13.0</td>
<td>1.0</td>
<td>20 to 30</td>
<td>posterior circulation</td>
</tr>
<tr>
<td>24</td>
<td>11.0</td>
<td>1.0</td>
<td>20 to 30</td>
<td>Posterior communicating artery</td>
</tr>
<tr>
<td>25</td>
<td>13.0</td>
<td>1.0</td>
<td>20 to 30</td>
<td>Proximal MCA</td>
</tr>
</tbody>
</table>

*Middle cerebral artery.
MOLINARI

FIGURE 3

Simple x-ray films immediately after embolization.
A. Shows the appearance of an occlusive embolus in the anterior cerebral artery.
B. Illustrates the typical location of an embolic cylinder in the middle cerebral artery foreshortened in this lateral view.

sured 1 mm, internal diameter, despite the wide range in body weights. Calculation of the internal diameter from the circumferential measurement added only a slight variability ranging from 0.9 mm to 1.1 mm. All measurements were made on fixed tissue in an attempt to standardize the error introduced by postmortem drying and shrinkage.

Cylinders of 1.66 mm diameter were found to be optimal for production of deep cerebral infarction. Emboli of this size were found in the middle cerebral artery, adjacent to the circle of Willis but proximal to the arborization at the Sylvian fissure. Segmental occlusions at this site undercut the anterior perforated substance, obstructing the origins of the lenticulostriate vessels. Major infarctions fell within the distribution of these penetrating arteries involving deep ganglia and the internal capsule. The cerebral cortex was largely spared in this type of embolic occlusion.

The six emboli of 1 mm diameter were less predictable as to the site of final occlusion. Two of these were found in the anterior cerebral circulation, two in the middle cerebral, and two in the posterior communicating artery. Deep infarctions were characteristically bland, attended rarely by punctate hemorrhages at their periphery.

Specimens with showers of small emboli showed multiple occlusions of meningocerebral vessels mainly on the side injected, but with occasional particles found in the contralateral hemisphere and in the posterior circulation. Infarctions in these brains were patchy, superficial, and hemorrhagic.

Discussion

The method described produces intracranial vascular occlusion in all cases when emboli were introduced into the internal carotid artery. Despite the cessation of flow in the cannulated internal carotid artery, foreign bodies, drugs, and contrast media are selectively delivered to the intracranial circulation because the pressure gradients favor flow through common carotid, external carotid, and maxillocarotid anastomosis into the lower-pressure internal carotid system. If the external carotid were ligated, the pressure gradient across the maxillocarotid anastomosis at the time of injection would favor flow from the internal carotid to the lower-pressure external carotid-maxillary artery system, reducing chances of successful intracranial embolism.

The lead-chrome pigment in the silicone rubber material permits immediate localization of the occlusion after embolization. Plain x-ray films differentiate between intracranial and...
extracranial occlusions, and identify the occluded branch of the intracerebral circulation with a high degree of accuracy. Figure 3A shows a cylinder in the anterior cerebral distribution. Figure 3B illustrates the typical x-ray appearance of a proximal middle cerebral artery embolism.

Although arteriography may be electively performed, this step is not needed to identify the site of occlusion. Upon postmortem examination, cursory inspection permits localization of large emboli because of the vivid pigmentation of the material (fig. 4). When showers of small emboli were used, the material was widely distributed throughout the cerebral hemisphere on the side injected. Small fragments on the medial surface and in meningeocerebral branches within sulci are not easily identified by gross inspection. Plain x-ray films of the specimen quickly and accurately define the number and distribution of all embolic particles. Because the material can be localized either by gross inspection or by the x-ray technique, correlation of the site of vascular occlusion with the area of cerebral infarction can be made in all cases. The polymer is not altered during the preparation of histological sections.

Early experiments in this series were done using emboli 5 mm long by 1 mm in diameter. Emboli of that diameter were those found in anterior cerebral and posterior communicating arteries. One embolus of this diameter occluded the proximal segment of the middle cerebral artery but fit the vessel loosely and produced only a small cystic lesion in the head of the caudate nucleus. In another specimen, a deep infarction in the lenticulostriate distribution, associated with a patent proximal middle cerebral artery, suggested distal migration of the embolus after a primary proximal occlusion. The embolus in this case was recovered from a branch of the middle cerebral artery overlying the parietal lobe cortex.

Emboli of 1.66 mm diameter consistently occluded the proximal middle cerebral artery probably because: (1) emboli of this size were too large to occupy the anterior cerebral artery, and (2) in the dog, as in the human, the middle cerebral artery is the natural continuation of the internal carotid. The sharp angulation required of the large cylindrical bolus in order to turn into the posterior communicating artery would be an unlikely occurrence. The inherent elasticity of the silicone rubber bolus maintains lateral pressure on the arterial wall despite postocclusive vasodilation. In most cases, this feature prevents migration of the cylinder distal to the original point of occlusion.

Occlusion of the posterior communicating artery or occlusion of the proximal middle cerebral artery produces a deep lesion of the internal capsule and basal ganglia. Because the meningeocerebral anastomoses of the cortex remain patent, blood perfuses the distal segments of the occluded artery in a retrograde fashion. Meyer and Denny-Brown showed that in order to produce cortical infarction in their animals, blood flow through collaterals had to be reduced along with obstruction in the primary source of blood supply.

The only arteries totally deprived of blood supply by this method are the penetrating vessels arising from the occluded segment. This
is seen best in a coronal section (fig. 5) showing the embolic cylinder underlying the origins of the lenticulostriate arteries. Because there are no precapillary anastomoses among penetrating vessels, ischemic infarction and coagulation necrosis develop only in deep structures, while cortical areas are relatively spared.

Smaller emboli occluding meningocerebral branches also produce infarction in the distribution of penetrating vessels. However, penetrating vessels of cortex have a shallow distribution, supplying gray matter and the immediately adjacent white matter structures. As demonstrated by Penry and Netsky in a study of meningocerebral artery occlusion in dogs, the subcortical infarctions were ischemic while gray matter infarctions were hemorrhagic, probably due to the myriad arterial anastomoses at the surface.

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