Temporary Occlusion of the Middle Cerebral Artery in the Monkey: Clinical and Pathological Observations

BY ROBERT M. CROWELL, M.D., YNGVE OLSSON, M.D., IGOR KLATZO, M.D., AND AYUB OMMAYA, F.R.C.S.*

Abstract:
Temporary Occlusion of the Middle Cerebral Artery in the Monkey: Clinical and Pathological Observations

The right middle cerebral artery (MCA) was exposed in monkeys via a retro-orbital microsurgical approach. In 43 animals a temporary occlusive clip was placed on the MCA origin for one to 24 hours. In 20 animals, the origin of the MCA was permanently occluded.

Clinical evaluation of the monkeys one to three days postoperatively showed that one to two-hour clipping caused no or mild neurological deficits, four-hour clipping caused mild to moderate deficits, six to eight-hour clipping caused moderate to severe deficits, and 24-hour clipping produced severe deficits or death, a result equivalent to that produced by permanent occlusion. Gross and microscopical evaluation of the brains showed that one to two-hour clipping usually caused no or mild damage, four-hour clipping caused mild to moderate damage (often with capsular sparing), and six to eight-hour clipping and 24-hour clipping produced severe extensive infarction not different from that caused by permanent occlusion. Six to eight-hour clipping and 24-hour clipping were associated with a high incidence of hemorrhagic infarction, but other clipping times were not. The results suggest that reestablishment of flow by surgical means within a few hours after MCA occlusion in selected patients might result in significant restoration of neurological function. If flow renewal were done within about four hours, an increased incidence of hemorrhagic infarction might be avoided.

ADDITIONAL KEY WORDS
focal cerebral ischemia stroke surgery microsurgical approach to MCA collateral blood supply

Introduction

Anatomical and physiological studies have demonstrated that abundant collateral blood supply exists distal to the circle of Willis. This collateral supply can compensate, at least in part, for the decrease in blood flow sustained after middle cerebral artery (MCA) occlusion in the macaque. In addition, cerebral tissue may be much more resistant to global ischemia than has previously been recognized. Such studies suggest that early restoration of flow after MCA occlusion might lead to improvement in appropriate stroke patients. This theoretical possibility has become more attractive since the development of feasible microsurgical technique for MCA thromboembolectomy and superficial temporal MCA anastomosis.

In evaluating the clinical role of flow renewal following MCA occlusion, it would be helpful to know how long focal cerebral ischemia may be tolerated before irreversible change develops. Several studies, most notably the classical work of Harvey and Rasmussen, were intended to determine the...
maximum tolerable duration of MCA occlusion, but this work was done without the aid of the dissecting microscope, and surgical trauma other than MCA occlusion probably caused some of the clinical and pathological findings. Recently Sundt and Waltz\textsuperscript{26} have described a microsurgical, retro-orbital approach to the MCA origin which avoids retraction of the brain, wide opening of the dural envelope, damage to the perforating vessels, and excess manipulation of the MCA. It seems likely that such a technique could provide information on the effects of temporary MCA occlusion minimally complicated by surgical trauma. We have, therefore, applied the method of Sundt and Waltz to produce temporary MCA occlusion in macaque monkeys.

The primary objectives of this study were (1) to ascertain the duration of occlusion required to produce functional and morphological changes comparable to those produced by permanent occlusion, and (2) to determine the maximal duration of occlusion which fails to produce significant functional and morphological changes.

**Methods**

**SURGICAL PROCEDURE**

Sixty-five monkeys (*Macaca mulatta*) were used for this study. Animals were given phencyclidine hydrochloride (Sernylan, 5 mg/kg IM), and small increments of sodium pentobarbital (up to 30 mg/kg IV or IP) were administered as needed during surgery. The operative procedures were carried out by one of us (RMC) under sterile (or occasionally semisterile) conditions. The Zeiss operating microscope was used to expose the origin of the right MCA via a retro-orbital approach.\textsuperscript{26} After the take-off of the MCA had been gently freed from its arachnoid investment, a control or test intervention was carried out: seven monkeys underwent division of the MCA between silver clips, 13 monkeys had permanent clipping with a Scoville aneurysm clip, two monkeys had no clipping whatever, and 43 monkeys had temporary clipping for one, two, four, six, eight, ten, or 24 hours (table 1). Occlusion of MCA by the clip was adjudged satisfactory only when the tip of the Scoville clip could be visualized beyond the far edge of the vessel and when anatomical continuity of the internal carotid artery (ICA) and anterior cerebral artery (ACA) appeared undisturbed. After clip removal, the dura was left open, and the galea and skin were closed with 3-0 interrupted silk or stainless steel sutures. In the first 20 monkeys, blood pressure was monitored with a femoral catheter and a Statham strain gauge. Mean pressure remained within the range of 95 to 110 mm Hg.\textsuperscript{26, 27}

**ANGIOGRAPHY**

Eighteen monkeys were studied with right brachial retrograde serial angiograms. These studies are to be reported elsewhere.\textsuperscript{28}

**CLINICAL EVALUATION**

Postoperatively animals were examined in their cages by one of us (RMC). Threat by the examiner elicited maximal clinical performance. A simple scheme for grading of deficits was employed (fig. 1).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Clinical Deficit</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
<td>Normal activities including normal climbing.</td>
</tr>
<tr>
<td>1</td>
<td>Mild</td>
<td>Abnormal climbing (slipping with contralateral limbs). Preference for use of ipsilateral hand. Mild</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Grade of clinical deficit</th>
<th>0</th>
<th>1-2</th>
<th>4</th>
<th>6-8*</th>
<th>24</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (none)</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1 (mild)</td>
<td>0</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2 (moderate)</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>6</td>
<td>2</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>3 (severe)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>4 (death)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>11</td>
<td>10</td>
<td>12</td>
<td>13</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

In this and subsequent tables, "p" indicates permanent MCA occlusion, and "d" means arterial division.

*One animal in this group was subjected to ten-hour MCA clipping; he sustained a grade 2 clinical deficit.
TEMPORARY OCCLUSION OF THE MIDDLE CEREBRAL ARTERY

A grade was assigned to each animal in the early postoperative period (day one to three) and weekly thereafter until sacrifice. In addition, most monkeys were examined at least once in a restraining chair one to two weeks postoperatively or just before sacrifice. Visual fields, motor strength, tone, reflexes, and response to pin prick were tested.

SACRIFICE
Monkeys were allowed to survive from two to 136 days following operation. Most animals were sacrificed at about three weeks (see Harvey and Rasmussen). After the administration of Sernylan, the descending thoracic aorta was cross-clamped, the right atrial appendage was opened, and transcardiac perfusion was carried out with two to three L of 4% paraformaldehyde in Millonig's phosphate buffer (pH 7.0). In a few animals, perfusion was accomplished via an aortic cannula after severing of the jugular veins. In most animals, Evans blue was administered one-half hour before sacrifice.

PATHOLOGICAL EVALUATION
After perfusion, brains were removed and additionally fixed by immersion in buffered paraformaldehyde solution. The brains from animals which died during the postoperative period were fixed by immersion in paraformaldehyde solution for two to three weeks. Each brain was cut into coronal slices 3 to 5 mm thick. After photography, slices from the area supplied by the MCA were immersed in a mixture of absolute alcohol, glacial acetic acid and formaldehyde. Slices were then embedded in paraffin, and sections ten microns thick were cut and then stained with cresyl violet or luxol fast blue-PAS.

Without prior knowledge of clinical findings, one of us (YO) evaluated the extent and type of infarction. Infarct size was estimated by macroscopical and microscopical inspection, and each infarct was graded according to a four-point scale (fig. 2).

<table>
<thead>
<tr>
<th>Grade</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No changes typical of infarction.</td>
</tr>
<tr>
<td>1</td>
<td>A few microscopical foci of necrosis not exceeding 3 mm in diameter.</td>
</tr>
<tr>
<td>2</td>
<td>Medium-sized infarct usually confined to the basal ganglia and internal capsule. Exceptionally infarction extends to a small area of the brain surface.</td>
</tr>
<tr>
<td>3</td>
<td>Large infarct in the central territory of the MCA. Occupies basal ganglia, internal and external capsules, claustrum, insula and extends broadly to the surface of the brain.</td>
</tr>
</tbody>
</table>

The infarcts were also classified as ischemic or hemorrhagic. The presence or absence of hemorrhagic infarction was determined from the

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FIGURE 1
Appearance of a monkey two days after temporary occlusion of the right MCA for four hours. This animal, which was typical for those occluded for four hours, showed minimal impairment of left hand movements during climbing but no other clinical deficits. He was classified as grade 1.
macroscopical appearance, since microscopical hemorrhages can be found in most infarcts.20, 80

Results

ANGIOGRAPHY
In almost every case with MCA clipping and subsequent right brachial arteriography, angiographical study demonstrated total MCA occlusion (fig. 3).

EARLY CLINICAL DEFICIT
The clinical consequences of MCA occlusion as assessed at one to three days postoperatively were closely related to the duration of clipping (table 1). Permanent occlusion (permanent clipping plus arterial division) produced a more severe deficit than did temporary clipping (one to two-hour, four-hour, six to eight-hour, and 24-hour clippings taken together). The outcome after one to two-hour clipping was not different from that after no treatment (non-ischemic control), but four-hour, six to eight-hour, and 24-hour clipping produced a more severe deficit than did no treatment. One to two-hour, four-hour, and six to eight-hour clipping produced fewer abnormalities than did 24-hour or permanent clipping. The results after permanent and 24-hour clipping were not different. Chi-square analysis has shown that each of these differences is statistically significant (p < 0.05).

MCA AT CLIPPING SITE
All clipping sites were examined grossly, and all MCAs were found to be free of internal thrombosis. Arteries subjected to temporary occlusion were often dilated and stained with Evans blue at the clipping site.

INCIDENCE AND SIZE OF CEREBRAL INFARCTIONS
In general, increasing duration of clipping was associated with increasing infarct size (table...
2). Permanent clipping and arterial division did not produce different pathological results in terms of infarct size. Permanent clipping and arterial division considered together caused a larger infarction than did temporary MCA clipping (one to two-hour, four-hour, six to eight-hour, and 24-hour taken together). Temporary clipping and permanent clipping both caused infarcts which were larger than those caused by no clipping. One to two-hour and four-hour clippings were different from permanent clipping in terms of infarct size, but six to eight-hour and 24-hour clippings were not different from permanent clipping in this respect. One to two-hour clipping produced an infarction larger than that produced by no clipping. Chi-square analysis showed these differences to be significant (p < 0.05).

TOPOGRAPHICAL DISTRIBUTION OF CEREBRAL INFARCTS

All infarcts were located within the area of supply of the MCA. None of the lesions were confined to the border zone between the territories of two major arteries ("watershed" infarcts). When the MCA was divided or permanently occluded, the size and the topographical distribution of ensuing lesions were rather uniform. These infarcts occupied the central territory supplied by the MCA. Infarcts produced by permanent MCA interruption involved the head of the caudate nucleus, the anterior limb and genu of internal capsule, lentiform nuclei (particularly putamen), external capsule, claustrum, insula, and white matter and cortex in the area around the sylvian fissure (fig. 2). Subependymal sparing

<table>
<thead>
<tr>
<th>Size of Infarction (grade)</th>
<th>Duration of occlusion (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
</tr>
</tbody>
</table>
Selectivity of the subcortical infarction. A. Low-power microscopical appearance of an infarction which involved the caudate nucleus and the putamen but spared most of the internal capsule and subependymal caudate. B. Gross appearance of a typical hemorrhagic infarction. Hemorrhage is limited to the caudate nucleus, the putamen, and the external capsule; internal capsule is spared. The area of ischemic infarction (as determined by microscopical examination) was slightly larger than the area involved with hemorrhage. C. Microscopical appearance of an infarction which severely damaged much of the caudate nucleus but spared a few layers of subependymal cells.

Lesions produced by temporary clipping were often limited to subcortical structures (table 2), and temporary clipping up to six to eight-hour duration frequently spared or only partly destroyed the internal capsule (fig. 4A, table 3). In these animals, the necrosis usually destroyed the middle part of the internal capsule, and the upper lateral and the lower medial parts were intact (eight of 11 animals).

Ischemic and hemorrhagic infarcts
When they occurred, hemorrhages almost always occupied only part of the infarcted area. Hemorrhages were usually confined to the basal ganglions (fig. 4B). With temporary occlusion for one to two hours or four hours and with permanent occlusion by clipping or division, infarctions were almost exclusively of the ischemic variety. When blood flow through the MCA was restored after six to eight-hour or 24-hour occlusion, ischemic and hemorrhagic infarcts were about equally common.

Brain swelling
Swelling of the operated cerebral hemisphere (as indicated by a shift of midline structures) was noticed only when the clipping had produced medium-sized or large infarcts (grade 2 to 3). Almost all animals with such lesions which were sacrificed during the first postoperative week showed considerable swelling of the brain, and about 50% of such animals taken during the second or the third week showed some swelling. None of the animals killed later than three weeks showed evidence of brain swelling.

Early clinical deficit and size of infarct
There was a roughly linear relationship between infarct size and early clinical deficit (fig. 5).

Clinical deficit and other pathological findings
Increasing early clinical deficit was loosely correlated with increasing involvement of internal capsule (fig. 5). Hemorrhagic infarctions and brain swelling were almost always associated with moderate to severe clinical deficits (grade 2 to 4).

Discussion
Clinical effects of temporary MCA occlusion
Careful clinical examination revealed that short-term temporary MCA clipping (one to eight hours) in monkeys leads to surprisingly mild impairment of gross neurological function. One to two-hour clipping leads to no change, four-hour clipping causes mild to moderate deficit, six to eight-hour clipping

| Table 3 |

<table>
<thead>
<tr>
<th>Internal capsule</th>
<th>Duration of occlusion (h)</th>
<th>1-2</th>
<th>4</th>
<th>6-8</th>
<th>24</th>
<th>p</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not infarcted</td>
<td></td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Partly infarcted</td>
<td></td>
<td>1</td>
<td>5</td>
<td>6</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Severely infarcted</td>
<td></td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>9</td>
<td>8</td>
<td>11</td>
<td>9</td>
<td>12</td>
<td>6</td>
</tr>
</tbody>
</table>
**TEMPORARY OCCLUSION OF THE MIDDLE CEREBRAL ARTERY**

**FIGURE 5**
Clinicopathological correlations. Left: The relationship between clinical deficit and size of infarction. The various levels of clinical and pathological grading are defined in the text. Note that most cases fall on the diagonal, indicating a good correlation between clinical deficit and size of infarction. See text for further discussion. Right: The relationship between clinical deficit and involvement of the internal capsule. See text for definitions. Note that many cases fall on the diagonal, indicating a loose correlation between clinical deficit and involvement of the internal capsule.

produces moderate to severe impairment, and 24-hour clipping leads to a severe or fatal outcome not different from that caused by permanent occlusion. In addition, the relation between clipping time and clinical deficit was rather constant.

**PATHOLOGICAL EFFECTS OF TEMPORARY MCA OCCLUSION**
In general, pathology showed that temporary MCA occlusion of one to four-hour duration usually caused no damage or small to medium-sized subcortical infarcts. One to two-hour clipping usually led to mild or no change, four-hour clipping caused moderate damage (often with capsular sparing), and six to eight-hour and 24-hour clipping produced severe damage not different in extent from that caused by permanent occlusion. Clipping time correlated well with the size of infarction and loosely with involvement of internal capsule (see also Sundt and Waltz). Our observations differ from the findings of other investigators who noted severe deficits and large infarcts after short clippings and inconsistency of lesion size after permanent clipping. Discrepancy between these investigations and the present study may be related to differences in species and surgical technique. Comparison of our data with the work of Sundt et al. suggests that cat and macaque have similar tolerances to MCA clipping, whereas squirrel monkey is much less resistant.

The localization of infarctions invites speculation as to some pathophysiological mechanisms involved in their genesis. The sparing of periventricular areas may be related to direct supply of substrate by CSF to nearby
Pathological examination revealed many hemorrhagic infarctions in animals clipped six to eight hours and 24 hours but not in those clipped one to two hours, four hours, and permanently. These data suggest that ischemia of more than four hours' duration may lead to vascular damage and subsequent hemorrhage on restoration of flow. In this connection it is known that MCA clipping and subsequent hypertension regularly lead to hemorrhagic infarction and this mechanism cannot be ruled out in our studies. In addition, several clinical studies have shown that removal of ICA or common carotid artery (CCA) occlusion in the early postictal period (up to two weeks) may be associated with a high incidence of hemorrhagic infarction. In these clinical studies, however, resumption of flow rarely occurred within four hours of the stroke ictus. Therefore, the relation between restoration of flow and hemorrhagic infarction may be similar in monkeys and man: restoration of flow by appropriate surgery within the first four hours might be associated with a low incidence of hemorrhagic infarction, and revascularization after greater delay may be associated with increased risk of hemorrhagic complications. Successful clinical results following very early removal of CCA-ICA or MCA occlusion are at least compatible with this concept. Careful clinicopathological correlations, with close attention to the interval to restoration of flow and to arterial blood pressure will be needed to decide this point in man.

TOLERANCE OF FOCAL CEREBRAL ISCHEMIA

Recent observations have suggested that, under special circumstances, even global ischemia lasting up to two hours may be borne by the central nervous system with relative impunity. This ability to withstand global ischemia may play a role in protecting monkey brain during MCA occlusion. In addition, there is abundant anatomical and physiological evidence that collateral blood supply protects the brain after occlusion of a major cerebral artery. This collateral supply is probably responsible, at least in part, for the tolerance of the monkey brain to temporary MCA clipping.

Anesthesia probably played a role in protecting monkeys from cerebral ischemia in the present study. Sodium pentobarbital probably protected cerebral tissue from ischemic damage, but this effect was probably attenuated by the decrease in total cerebral flow induced by the drug. Obviously, a study of the effects of temporary MCA occlusion on monkey brain unaffected by drugs would be desirable, but technical problems will make such a study difficult.

Leakage of blood past an incomplete occlusion of the MCA might be postulated as a factor which would protect monkey brain during MCA clipping. Against this possibility, angiographical studies have demonstrated that Scoville clips insure total MCA occlusion when properly placed.

IMPLICATIONS FOR CLINICAL PRACTICE

The experimental data reported here suggest that removal of MCA occlusion within a few hours of the stroke ictus might lead to useful restoration of function in some clinical cases with suitable collateral circulation. Limited neurosurgical experience with prompt MCA thromboembolectomy supports this concept. It may be that angiography and/or regional cerebral blood flow studies could help in selecting cases with suitable focal lesions and collateral blood supply for such surgical therapy. In addition, the results suggest that during neurosurgical procedures temporary MCA occlusion lasting up to several hours might be tolerated in patients with good collateral supply. In such cases, the protective effect of general anesthesia could also be utilized, and preoperative studies might help determine the adequacy of collateral blood flow. The neuropathological data suggest that if restoration of blood flow is accomplished within about four hours after occlusion, an increased incidence of hemorrhagic cerebral infarction might be avoided.

Acknowledgment

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