Experimental Cerebral Infarction

Part 1: Production of Thalamic Infarction in Dogs

Takashi Yoshimoto, M.D., Tetsuya Sakamoto, M.D., and Jiro Suzuki, M.D.

SUMMARY Difficulties in achieving focal temporary cerebral ischemia in experimental animals have delayed study of the prevention and treatment of cerebral infarction. We have succeeded in producing focal cerebral infarction by temporary occlusion of brain arteries. Infarction confined to the anterior portion of the thalamus was obtained by simultaneous occlusion of the 4 cerebral arteries: internal carotid, anterior cerebral, middle cerebral and posterior communicating arteries for 60-120 minutes. This experimental model in dogs is unique, since thalamic infarction can be produced with high frequency, and the dogs can be kept alive and managed for sufficient periods after temporary artery clipping. With this model it is possible to investigate cerebral infarction not only from the pathophysiological viewpoint, but also from the viewpoint of prevention and treatment of cerebral infarction in man.

DEVELOPMENTS in surgical treatment of cerebrovascular diseases have accelerated the necessity for solving the problem of cerebral infarction. In studies on cerebral occlusive diseases, little is known about the correlation between the timing of surgical intervention, surgical results and the useful restoration of function. Although cerebral infarction has been investigated by various approaches, sufficient information on these points is still not available. In experiments of total circulatory arrest, 1-5 even though cerebral ischemia is induced, severe brain damage or massive damage of the entire body limits the observation time. Furthermore, the experiments disregard the existence of cerebral collateral circulation which plays an important role in the pathophysiology of cerebrovascular diseases. In experiments of permanent occlusion of a brain artery, 6-10 even when the infarctions were regularly induced, it has been impossible to resolve the problems of postischemic ruffle. In experiments on occlusion of middle cerebral artery in monkeys, 11-13 infarction could not be regularly induced by clipping or short-temporary occlusions. Furthermore, after prolonged-temporary clipping clinicopathological examinations after ischemia were often obstructed by severe postoperative deterioration or death of the experimental animal.

Several experiments to produce cerebral infarction by occlusion of the middle cerebral artery in dogs have been carried out. 14-18 We could not obtain predictable thalamic infarction, chiefly because of complex and abundant collateral circulation. 14-18 We succeeded in producing focal infarction confined to the anterior portion of the thalamus by simultaneous occlusion of all four cerebral arteries (internal carotid, anterior cerebral, middle cerebral, and posterior communicating arteries) for 60-120 minutes. In this paper, the method of inducing thalamic infarction in the dog is presented.

Methods

Surgical Procedure

Forty-three adult mongrel dogs weighing about 10 kg were anesthetized with intravenous sodium pentobarbital (Nembutal) 35 mg/kg. After the removal of the zygomatic arch, the sphenoidal ridge was rongeuried approximately 3 mm in the anterior and 10 mm in the posterior direction. In order to avoid or minimize artificial brain damage by occlusion of arteries, utmost care was taken to rongeur the middle fossa as far as possible.

After the dural incision, cerebrospinal fluid was gently aspirated. When the arachnoid was opened near the optic chiasma, the brain showed marked shrinkage disclosing the termination of the internal carotid artery without retraction of the brain. Despite the aspiration of cerebrospinal fluid, retraction of the temporal lobe base was necessary to expose the distal portion of the posterior communicating artery. Occasionally, because of this, temporal lobe infarction was combined with thalamic infarction. Sham operations were performed to evaluate the other infarctions. Only thalamic infarctions were included in this study since the relations between the surgical procedures and the temporal lobe infarctions could not be clearly judged.

After adequate exposure cerebral arteries were simultaneously occluded with Scoville aneurysmal clips using the Zeiss operating microscope. They were the internal carotid artery just proximal to the junction of the posterior communicating artery, the middle and the anterior cerebral arteries at their origins, respectively, and the distal portion of the posterior communicating artery (fig. 1). In all dogs the occluded arteries were carefully freed from the arachnoid membrane. Three dogs acting as controls had no clips applied. Forty dogs had temporary clipping for periods of 30, 60 and 120 minutes. Subsequently, the clips were removed, a dural substitute was implanted and the operative wound was closed. The operative procedures were carried out under sterile conditions. Blood pressure was monitored with a femoral catheter, and remained within the normal range during the operation.

Results

Pathological Evaluation

All dogs were sacrificed on the 7th postoperative day. After brain fixation, coronal slices of approximately 5 mm in thickness were made for pathological studies. Infarctions confined to the anterior portion of the thalamus were observed only from brain slices made 5 mm behind the optic chiasma (fig. 2). In regions excluding the thalamus, except...
for some foci of infarction irregularly seen in the operated temporal cortex, no infarctions were evident in the cortex, basal ganglia or internal capsule.

Microscopic examination showed typical ischemic infarction. The edges of the infarctions were clearly distinguished. In the infarcted area there was marked vascular proliferation and extensive infiltration with microglial cells, which were actively phagocytic (fig. 3).

The size of the infarctions in thalamus were estimated microscopically from slices made 5 mm behind the optic chiasma, and each infarction was graded according to a 4-point scale: grade 0: no infarction observed in the thalamus, grade 1: small (less than half) infarction of the thalamus, grade 2: infarction affecting about half of the thalamus, grade 3: infarction affecting more than two-thirds of the thalamus (fig. 4).

The control group had no infarctive changes. The group with temporary occlusion for 30 minutes showed minimal changes. However, in the group with occlusion for 60 minutes, 6 of 10 dogs had marked infarction of grade 2 or 3. Marked infarctions were found in 13 of 20 dogs with temporary clipping lasting 120 minutes (table 1).

Discussion

Several authors have reported successful production of cerebral infarction in dogs subjected to permanent or temporary arterial clipping, but it has been generally difficult to induce constant cerebral infarction. Our preliminary studies in 43 dogs sacrificed 1 week later showed no typical infar-
tions following temporary occlusion of the middle cerebral artery. As shown in table 2, cerebral infarction was not regularly obtained by temporary occlusions. Although all 3 dogs in the permanent occlusion group showed marked infarctions of grade 1 or 2, the size and the location of the infarctions were different in each dog. Further, to reduce gross cerebral blood flow, 14 of the 19 dogs subjected to occlusion of the MCA for 2 hours were subjected to permanent ligations of the common carotid and vertebral arteries. However, as shown in table 3, infarctions were not observed regularly. As shown in Part 2 of this series — Electroencephalographic changes — the cortical EEG tracings of the ipsi- and contralateral sides were identical following occlusion of the MCA, ICA, ACA and PcomA. The results illustrate the difficulties of inducing cerebral infarctions regularly by occlusion of the MCA in dogs.

To examine the effectiveness of collateral circulation to the middle cerebral artery, the artery was cut near the origin of the circle of Willis and a small catheter was inserted distally to measure blood pressure. Immediately after the insertion of the catheter, blood pressure in the distal middle cerebral artery averaged about 60 mm Hg and was unchanged for up to 2 hours. While the origin of the middle cerebral artery remained occluded, the brain was perfused with an injected suspension of carbon on the side of the occluded middle cerebral artery. A non-perfused area could not be seen. The gray matter in both hemispheres was evenly colored black.

Cerebral collateral circulation prevents the production of constant cerebral ischemia in laboratory animals, including monkeys. In experiments with monkeys, investigators have indicated minimum neurological deficits and no infarction after brief middle cerebral artery clipping. Meyer stated, "to produce a degree of impairment as severe as that associated with permanent occlusion, it is necessary to occlude the vessel for at least 50 minutes. However, a few animals with permanent occlusion of the middle cerebral artery had transient hemiplegia without severe infarction." Crowell et al. reported "that temporary middle cerebral artery occlusion of 1 to 2 hours duration usually caused no damage or only a small sized subcortical infarction."

After numerous trials and errors, we have succeeded in producing focal cerebral infarction in dogs by temporary occlusion of brain arteries. Thalamic infarction was induced regularly by simultaneous occlusion of all 4 cerebral arteries. In these experiments it is probable that many fine perforating arteries were occluded by 4-artery clipping and this may accounts for the appearance of cerebral infarction in the anterior portion of the thalamus. Although impairment of gross neurological function was not evident, marked infarctions of grade 1 or 3 were obtained in approximately two-thirds of the dogs subjected to the temporary occlusion for 60 minutes and 120 minutes.

This thalamic infarction model in dogs has advantages since marked infarction can be made with high frequency, and the animals can survive for sufficient intervals after temporary occlusions, making it possible to investigate cerebral infarction pathologically as well as to investigate methods of prevention and treatment.

References

### Table 1 Correlation Between Thalamus Infarction and Length of Occlusion in Dogs

<table>
<thead>
<tr>
<th>Thalamus infarction</th>
<th>Grade 0</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occlusion time</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham operation</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>30 minutes</td>
<td>7</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>60 minutes</td>
<td>4</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>120 minutes</td>
<td>4</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td>20</td>
</tr>
</tbody>
</table>

Grade 0: no infarction in thalamus. Grade 1: small infarctic foci in thalamus. Grade 2: infarction affecting about half of thalamus. Grade 3: infarction affecting more than two-thirds of thalamus.

### Table 2 Cerebral Infarction Following MCA Occlusion

<table>
<thead>
<tr>
<th>Time</th>
<th>G (0)</th>
<th>G (1)</th>
<th>G (2)</th>
<th>G (3)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operation</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>1 hour</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>2 hours</td>
<td>9</td>
<td>7</td>
<td>3(1)</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>2.5 hours</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>3 hours</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Permanent</td>
<td>0</td>
<td>0</td>
<td>2(1)</td>
<td>1(1)</td>
<td>3</td>
</tr>
</tbody>
</table>

G 0: No infarction; G 1: Microscopie infarctic foci; G 2: Infarction less than 5 mm; G 3: Infarction more than 5 mm; (1) Infarction including basal ganglia.

### Table 3 Cerebral Infarction with 8 Hours MCA Occlusion

<table>
<thead>
<tr>
<th>Occlusion methods</th>
<th>G (0)</th>
<th>G (1)</th>
<th>G (2)</th>
<th>G (3)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCA one clip</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>MCA divided clip</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>MCA divided clip + C.L.</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>MCA divided clip + both C.L.</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>MCA divided clip + 4 vessels L.</td>
<td>2</td>
<td>0</td>
<td>1(1)</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

CLT = contralateral; C. L. = common carotid ligation.
Experimental Cerebral Infarction

Part 2: Electroencephalographic Changes Produced by Experimental Thalamic Infarction in Dogs

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SUMMARY Previously, one of the authors developed a reliable experimental model in dogs for producing cerebral infarction. The authors have now used this model to study the electroencephalographic changes produced by experimental thalamic infarction. The model was useful in predicting ischemic regions or infarction.

Methods

Adult mongrel dogs were used in these experiments. Intravenous pentobarbital (35 mg/kg) was used for general anesthesia. The airway was secured with an oral endotracheal tube with spontaneous respiration. After fixing the head of the dog on a stereotaxic apparatus, the airway was secured with an oral endotracheal tube. After feeding the dog with a catheter and a reference electrode was placed in the paranasal sinus (fig. 1). These were immobilized with dental cement. The animal was then removed from the stereotaxic apparatus and an intracranial vascular occlusion was produced surgically as described elsewhere.

The EEG tracings were directly observed on the oscilloscope and simultaneously recorded on a data recorder which fed them to a signal processor (San-ei Sokki, 7T07) for power spectrum analysis, and recording on an X-Y recorder.

Experiment 1. Repeated Short Term Occlusion

We occluded 4 cerebral arteries simultaneously (internal carotid, middle cerebral, anterior cerebral, and posterior cerebral) with clamps and released them after 5 minutes. This procedure was repeated several times at 20 minute intervals.

Experiment 2. 7 Day Follow Up

In Experiment 2 we used only those animals whose depth EEG recording from the thalamus showed diminution of fast waves with an attenuation of voltage on the side of the surgical occlusion. The 4 arteries were occluded for 2 hours. Serial EEGs were recorded for approximately 10 minutes following an intravenous dose of pentobarbital (15 mg/kg) on days 3, 5, and 7 after the surgery.

Autopsy and histological examination were made on the 7th day after surgery. The sizes of the infarctions in the thalamus were estimated microscopically, and each infarction was graded according to a 4-point scale: grade 0: no infarction in the thalamus, grade 1: small infarction foci (less than 0.5 mm), grade 2: medium-sized infarctions (0.5-1.5 mm), and grade 3: large infarctions (over 1.5 mm).

EEG increased detection of experimental cerebral infarction, and was useful in predicting ischemic regions or infarction.
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