Prediction of Stroke Before and After Unilateral Occlusion of the Common Carotid Artery in Gerbils

Masayasu Matsumoto, MD, Takao Hatakeyama, MD, Fumiharu Akai, MD, Joan M. Brengman, BA, and Takehiko Yanagihara, MD

A method was developed to predict the severity of cerebral ischemia before permanent occlusion of a common carotid artery in gerbils by observing the diameter and appearance of the artery after temporary occlusion and observing clinical signs after permanent occlusion. The severity of cerebral ischemia was confirmed by a sensitive immunohistochemical method and measurement of focal cerebral blood flow after 30 minutes' ischemia. All gerbils with >40% reduction of the diameter and a white arterial margin distal to temporary occlusion developed severe neurologic signs following permanent occlusion, but no gerbils with reduction of <30% and a red arterial margin developed neurologic signs. With the cumulative neurologic score, gerbils could be divided into classes with no, mild, moderate, and severe symptoms, mostly after 10 minutes. Severely symptomatic gerbils were identified in 3 minutes. Extensive ischemic damage was observed in severely symptomatic gerbils, but no immunohistochemical lesion was detected in mildly symptomatic gerbils. Cerebral blood flow was markedly reduced in severely symptomatic gerbils but more selectively reduced in the cortical structures of moderately symptomatic gerbils. This prediction method is useful for investigating early cerebral ischemia and for evaluating the effectiveness of pharmacologic agents. (Stroke 1988; 19:490-497)

In the past 3 decades, a large number of animal models have been used for investigating cerebral ischemia. Since Levine and Payan observed cerebral infarction after occlusion of a common carotid artery (CCA) in gerbils, this species has been used frequently because of its convenience in producing cerebral ischemia. However, this experimental model has drawbacks; we must rely on clinical manifestations to identify the affected gerbils, and the severity of cerebral ischemia and damage may vary among affected gerbils. To overcome these drawbacks and to make this experimental model more suitable for investigating early cerebral ischemia and for evaluating the effectiveness of pharmacologic agents, we established a method to predict the severity of cerebral ischemia before sustained arterial occlusion and to determine the severity within 3–5 minutes after arterial occlusion.

Materials and Methods

Sixty-four Mongolian gerbils (Meriones unguiculatus) weighing 60–80 g of either sex were used for this study, 48 gerbils for the prediction of stroke and the immunohistochemical investigation and 16 gerbils for the measurement of focal cerebral blood flow (CBF). Gerbils were kept in the animal quarters with food and water ad libitum before temporary or permanent occlusion of the CCA.

Prediction of Stroke

Each gerbil was anesthetized with 100 mg/kg i.p. ketamine hydrochloride. After midline skin incision, the right CCA was exposed and separated from the surrounding structures (such as the vagus nerve and the jugular vein) under an operating microscope, and a white rubber strip and a 5-0 silk suture were passed under it (Figure 1). Two 6-0 silk sutures were passed around the 5-0 silk suture, and the latter was loosely looped. After calibrating the diameter of the CCA to 10 units of the microscale in the microscope (Figure 1), the CCA was occluded for <30 seconds by tightening the 5-0 silk suture and releasing it quickly by pulling the two 6-0 silk sutures in opposite directions. During temporary occlusion, diameter of the CCA distal to the occlusion was measured and the color of the arterial edge was recorded as red (Type 1), pink (Type 2), or white (Type 3). There were some arteries with appearances between Types 1 and 2 or between Types 2 and 3. After removal of the sutures, the incision in the neck was closed and each gerbil was allowed to recover. No abnormal behavior was observed during the 3-hour recovery period in any gerbil.

Assessment of Neurologic Status

Two to seven days after the above prediction study, each gerbil was lightly anesthetized with ether inhalation, and the right CCA was exposed again and doubly ligated when the gerbil began to recover from anesthesia. The neurologic status of each gerbil was observed closely and scored at 3 and 5 minutes and every 5 minutes thereafter for a total of 30 minutes. The person who carried out permanent occlusion and scored neurologic status was blinded to the outcome of the
TABLE 1. Neurologic Signs for Scoring Clinical Condition at Torsion of Neck

<table>
<thead>
<tr>
<th>Sign</th>
<th>Neurologic score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disturbance of consciousness</td>
<td></td>
</tr>
<tr>
<td>Drowsiness</td>
<td>1</td>
</tr>
<tr>
<td>Semicoma</td>
<td>2</td>
</tr>
<tr>
<td>Coma</td>
<td>7</td>
</tr>
<tr>
<td>Ipsilateral circling</td>
<td>1</td>
</tr>
<tr>
<td>Torsion of neck</td>
<td>1</td>
</tr>
<tr>
<td>Contralateral ptosis</td>
<td>2</td>
</tr>
<tr>
<td>Contralateral hemiparesis</td>
<td>1</td>
</tr>
<tr>
<td>Seizure</td>
<td>2</td>
</tr>
<tr>
<td>Maximal total</td>
<td>7</td>
</tr>
</tbody>
</table>

prediction study. For prompt neurologic assessment and scoring, six neurologic signs were used (Table 1). Disturbance of consciousness was divided into drowsiness (paucity of movement), semicoma (no movement but preserved righting reflex), and coma (loss of righting reflex). Contralateral hemiparesis was tested by placing each gerbil on a tilting board. Based on our previous experience that semicoma and seizure occurred only in severely affected gerbils, a score of 2 was given for each phenomenon. Thus, the maximal score at each observation period was 7. Coma was rare within 30 minutes after unilateral CCA occlusion. When it occurred, the maximal score of 7 was automatically given for that observation period. Based on the total score for each observation period, the gerbils were classified as asymptomatic (total score 0), mildly symptomatic (total score 0–1), moderately symptomatic (total score 2–3), or severely symptomatic (total score 4–7) (Table 2). Although the score was 0, gerbils with ipsilateral ptosis, equivocal drowsiness, and/or mild intermittent ipsilateral circling were separated from asymptomatic gerbils and classified as mildly symptomatic. We also calculated the cumulative score by adding the total score of each observation period for each gerbil to measure the morbidity of a given period of observation (Table 2). Based on the cumulative scores, gerbils were classified as asymptomatic (cumulative score 0), mildly symptomatic (cumulative score 0 to 2N–1), moderately symptomatic (cumulative score 2N to 4N–1), or severely symptomatic (cumulative score 4N to 7N) at the Nth observation period. The reason for a cumulative score of 0 for some mildly symptomatic gerbils has been explained.

Immunohistochemical Procedure

Thirty minutes after permanent CCA occlusion, each gerbil was decapitated after brief ether inhalation and the brain was promptly removed, divided into coronal sections, fixed in ethanol-5% acetic acid, and embedded in paraffin. The immunohistochemical reaction for tubulin was carried out with the 5-μm brain sections corresponding to the stereotaxic section by using the peroxidase-antiperoxidase method. The antiserum for tubulin from gerbil brains has been produced in our laboratory. Harris’ hematoxylin was used for counterstaining. An adjacent section was stained with hematoxylin and eosin for comparison. Each brain section was examined by two or three investigators. The first investigator was not blinded to the neurologic status; the second investigator examined the sections blindly. If there was disagreement between them, the third investigator examined the section blindly. There were only several brain sections that required the third investigator. Loss of the immunohistochemical reaction in the neuropil, neuronal perikarya, and dendrites was used as criteria for ischemic damage. For topographic presentation of the extent of ischemic damage, areas with loss of the immunohistochemical reaction were traced on a map of the coronal brain section, and the maps from four brains were superimposed.

To rule out possible ischemic damage caused by temporary occlusion of a CCA for <30 seconds, four gerbils that were predicted to be severely symptomatic were allowed to live for 1 week after temporary occlusion without permanent ligation, and each brain

![Diagram of procedure for temporary ligation as viewed through operating microscope. A 5-0 silk suture is around common carotid artery for temporary ligation. Two 6-0 sutures were used to release temporary ligation promptly by pulling them in opposite directions. Three different appearances of common carotid artery distal to temporary occlusion are also shown. Margin of collapsed arterial wall was red in Type 1, pink in Type 2, and white in Type 3.

FIGURE 1. Schematic presentation of procedure for temporary ligation as viewed through operating microscope. A 5-0 silk suture (closed arrows) is around common carotid artery for temporary ligation. Two 6-0 sutures (open arrows) were used to release temporary ligation promptly by pulling them in opposite directions. Three different appearances of common carotid artery distal to temporary occlusion are also shown. Margin of collapsed arterial wall was red in Type 1, pink in Type 2, and white in Type 3.

TABLE 2. Total and Cumulative Neurologic Score for Clinical Classes of Gerbils With Permanent Common Carotid Artery Occlusion

<table>
<thead>
<tr>
<th>Class</th>
<th>Total score after</th>
<th>Cumulative score after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>single rating</td>
<td>multiple ratings</td>
</tr>
<tr>
<td>Severely symptomatic</td>
<td>4 to 7</td>
<td>4N to 7N</td>
</tr>
<tr>
<td>Moderately symptomatic</td>
<td>2 to 3</td>
<td>2N to (4N–1)</td>
</tr>
<tr>
<td>Mildly symptomatic</td>
<td>0 to 1</td>
<td>0 to (2N–1)</td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

N, number of observations 3 to 30 minutes after occlusion as indicated in Figure 5.
was examined immunohistochemically. Since the immunohistochemical reaction for astroprotein or glial fibrillary acidic protein (GFAP) (Dako Corp., Santa Barbara, California) is sensitive for detecting subtle evidence of previous ischemic damage even in areas without loss of the reaction for tubulin, the immunohistochemical reactions for tubulin and GFAP were used for these four gerbils.

**Measurement of Focal Cerebral Blood Flow**

The quantitative autoradiographic technique described by Sakurada et al. was used to measure CBF. Sixteen gerbils, four in each neurologic status class, were predicted by temporary occlusion of the right CCA as before. After a few days, each gerbil was anesthetized with 100 mg/kg i.p. ketamine hydrochloride, and the tail artery and the right saphenous vein were cannulated with PE-10 polyethylene catheters filled with 50 IU heparin/ml saline and sealed. The right CCA was reexposed and looped with a 5-0 silk suture for future ligation, and the wound was closed. Several hours later, each gerbil was lightly anesthetized with ether inhalation and the right CCA was ligated as the gerbil emerged from brief anesthesia.

Each gerbil was observed for the presence or absence of neurologic manifestations as before until 25 minutes after CCA occlusion, reanesthetized with 50 mg/kg i.p. ketamine hydrochloride, and placed in the supine position. Measurement of mean arterial pressure and blood sampling (100 μl) for gas analysis were carried out through the arterial cannula, and 30 μCi [14C]iodoantipyrine (Amersham, Arlington Heights, Illinois) in 0.8 ml physiological saline was infused through the saphenous vein for 1 minute beginning 30 minutes after CCA occlusion, while 10-18 drops of arterial blood were collected. At the end of the infusion, the brain was obtained by decapitation, frozen quickly in isopentane chilled to -50° C, and sectioned (20 μm) in a cryostat at -20° C. Each coronal section was transferred to a cover glass and immediately dried at 60° C. An autoradiogram was prepared by placing a series of cover glasses with brain sections in contact with a Kodak OM-1 film (Rochester, New York) for 12 days with calibrated [14C]methacrylate standards (Amersham). An autoradiogram was measured using a densitometer (model PPD, Sargent-Welch, Skokie, Illinois) with a 0.2-mm aperture. Based on our previous investigations, 24 anatomic sites were selected for measurement of focal CBF to represent vulnerable and less vulnerable areas. Focal CBF was computed according to Sakurada et al. Tukey’s method was used for statistical analysis.

**Results**

**Prediction of Stroke by Temporary Arterial Occlusion**

As shown in Figure 2, there was a clear correlation between neurologic outcome and reduction in the size of the margin of the distal CCA. No gerbils with a <30% reduction of the size and a red margin developed any neurologic signs after permanent occlusion. On the other hand, all gerbils with a >40% reduction of the diameter and a white margin developed the full constellation of neurologic signs. Mildly or moderately symptomatic gerbils showed a size reduction and margin appearance between severely symptomatic and asymptomatic gerbils.

**Temporal Profile of Neurologic Signs**

As shown in Figure 3, drowsiness, circling, and contralateral hemiparesis were observed in moderately and severely symptomatic gerbils as early as 3 minutes after CCA occlusion, and contralateral ptosis was observed only in severely symptomatic gerbils. Torsion of the neck and seizure distinguished the severely symptomatic gerbils from the others after 7 minutes following CCA occlusion. Mildly symptomatic gerbils showed ipsilateral circling and contralateral hemiparesis but not contralateral ptosis, torsion of the neck, or seizure for 30 minutes.

Accuracy of the cumulative score at 3, 5, and 10 minutes for predicting the outcome at 30 minutes is shown in Figure 4, and the mean cumulative scores of the gerbils in different neurologic status classes at each observation period are shown in Figure 5. After 3 minutes, we could identify severely symptomatic gerbils if they had a cumulative neurologic score of 4. After 5 minutes, we could identify the majority of gerbils that would develop severe or mild neurologic signs at 30 minutes by a cumulative score of 7–10 or 1–2, respectively. However, there was considerable overlapping of the clinical severity between cumulative scores of 3 and 6 at 5 minutes. All gerbils with a cumulative score of >10 at 10 minutes were severely symptomatic at 30 minutes, and all but one gerbil with a cumulative score of 5–9 at 10 minutes became moderately symptomatic at 30 minutes, while those with a cumulative score of <5 became mildly symptomatic at 30 minutes. Cumulative neurologic score at 30 minutes ranged from 36 to 45 for severely symptomatic gerbils and from 14 to 25 for moderately symptomatic gerbils, while cumulative score for mildly symptomatic gerbils ranged from 0 to 10 at 30
minutes. Those with a cumulative score of 0 in the mildly symptomatic class had subtle clinical manifestations (see "Materials and Methods").

**Immunohistochemical Investigation**

Frequency of the pathologic lesions detected by the immunohistochemical reaction and hematoxylin and eosin staining in various brain regions 30 minutes after CCA occlusion is shown in Table 3. In moderately symptomatic gerbils, the regions known to be vulnerable to ischemia [such as CA1 and CA2 of the hippocampus and layer III (IV) of the cerebral cortex] were all affected. In severely symptomatic gerbils, even regions known to be less vulnerable (such as CA3 of the hippocampus, thalamus, and caudoputamen) were affected. No immunohistochemical lesion evolved in mildly symptomatic or asymptomatic gerbils within 30 minutes. Hematoxylin and eosin staining was less sensitive in detecting early ischemic lesions. Immunohistochemical lesions in the moderately and severely symptomatic classes are topographically presented in Figure 6. While the vulnerable regions were consistently affected, lesions in the less vulnerable regions were heterogeneous. As gerbils became more severely symptomatic clinically, the caudoputamen and thalamus were more affected immunohistochemically. In the most severely symptomatic class, regions close to the midline (such as the hypothalamus and habenular nucleus) and sometimes even regions on the opposite side became affected.

No lesion was detected in gerbils subjected to temporary occlusion of the right CCA for < 30 seconds and subsequent reperfusion for 1 week when they were examined with the immunohistochemical reaction for tubulin or GFAP or with hematoxylin and eosin staining.

**Measurement of Focal Cerebral Blood Flow**

The physiologic condition of 16 gerbils just before measurement of CBF is shown in Table 4. There were no significant differences in any parameter among the four neurologic status classes.

The intracerebral distribution of [14C]iodoantipyrine in a severely symptomatic and a moderately symptomatic gerbil are shown in Figure 7. In severely symptomatic gerbils, distribution of the radiotracer was markedly and widely reduced in the hippocampus, cerebral cortex, caudoputamen, and thalamus except for the region close to the midline. On the other hand, distribution of the radiotracer in moderately symptomatic gerbils showed microheterogeneity even in the hippocampus and cerebral cortex, and distribution in the caudoputamen and thalamus was relatively well preserved. In mildly symptomatic gerbils (not shown), slight reduction could be observed in the cerebral cortex and hippocampus.

The profile of mean focal CBF in 34 subregions is shown in Figure 8. In asymptomatic gerbils, mean ± SEM CBF ranged from 60.0 ± 6.2 ml/100 g/min in the septal nucleus to 158.5 ± 23.5 ml/100 g/min in the median nucleus of the thalamus. Although mild re-
production (5–30%) of mean CBF existed in the mildly symptomatic gerbils, no value was significantly different from the corresponding value of asymptomatic gerbils. Marked reduction of focal CBF was observed in moderately and severely symptomatic gerbils compared with asymptomatic gerbils ($p<0.05$) in most subregions except those close to the midline. The differences between moderately and severely symptomatic gerbils were not significant in general. In the vulnerable subregions, such as the subiculum-CA1 (2) in Figure 8 and CA2 (5) of the hippocampus and the layer III (IV) of the cerebral cortex (12, 15, 18, and 21), mean CBF ranged from 9.0 to 18.5 ml/100 g/min in moderately symptomatic gerbils and from 0.3 to 7.0 ml/100 g/min in severely symptomatic gerbils. In less vulnerable subregions, such as the lateral part of the caudoputamen (31 and 32) and the ventral nucleus of the thalamus (24), mean CBF ranged from 30.8 to 36.3 ml/100 g/min in moderately symptomatic gerbils but...
from 1.3 to 1.8 ml/100 g/min in severely symptomatic gerbils. Reduction of focal CBF was also noted on the left side, especially in the midline structure of severely symptomatic gerbils, reaching ≤50% (p < 0.05) of the corresponding values in asymptomatic gerbils.

### Discussion

Unilateral occlusion of the CCA in Mongolian gerbils has been used widely to produce experimental cerebral ischemia because of the relative simplicity of the procedure. Its unilaterality and clinical signs are closer to the common manifestations of cerebral ischemia in humans than bilateral global ischemia. However, the necessity of clinical observation for identification of symptomatic gerbils and the variability in severity of cerebral ischemia are drawbacks. In our investigation, we attempted to establish a method to predict severity before sustained arterial occlusion and to confirm it soon afterward. By combining observation of the diameter of the distal CCA and appearance of the arterial margin, we predicted neurologic outcome before permanent occlusion. Our results are comparable with those of Laas, who measured arterial blood pressure distal to the CCA ligation in gerbils and correlated it with the clinical and histopathological outcome. Neurologic conditions following unilateral CCA occlusion in gerbils have been studied by several investigators, and the clinical signs of cerebral ischemia have been detected 5 minutes after CCA occlusion. With our method, we were able to identify severely affected gerbils as early as 3 minutes after permanent CCA occlusion and were able to separate gerbils into four clinical classes, most

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**TABLE 4. Physiologic Conditions of Gerbils Used for Measurement of Cerebral Blood Flow**

<table>
<thead>
<tr>
<th>Class</th>
<th>n</th>
<th>Hematocrit (%)</th>
<th>MABP (mm Hg)</th>
<th>pH</th>
<th>Paco2 (mm Hg)</th>
<th>Paco2 (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asymptomatic</td>
<td>4</td>
<td>46.5±1.4</td>
<td>97.8±3.9</td>
<td>7.29±0.01</td>
<td>79.5±4.1</td>
<td>41.4±1.2</td>
</tr>
<tr>
<td>Mildly symptomatic</td>
<td>4</td>
<td>53.3±3.9</td>
<td>87.5±9.9</td>
<td>7.30±0.03</td>
<td>94.0±6.5</td>
<td>36.7±1.3</td>
</tr>
<tr>
<td>Moderately symptomatic</td>
<td>4</td>
<td>48.0±3.5</td>
<td>83.5±8.0</td>
<td>7.29±0.06</td>
<td>90.5±4.3</td>
<td>36.9±3.6</td>
</tr>
<tr>
<td>Severely symptomatic</td>
<td>4</td>
<td>49.8±1.0</td>
<td>104.8±4.6</td>
<td>7.28±0.02</td>
<td>85.3±6.5</td>
<td>39.3±3.2</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. MABP, mean arterial blood pressure. No significant difference was found with Tukey's test.

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**FIGURE 7.** Representative autoradiograms showing focal cerebral blood flow in coronal sections including frontoparietal cortex and caudoputamen (A and B) and parietal cortex, hippocampus, and thalamus (C and D) in severely symptomatic (A and C) and moderately symptomatic (B and D) gerbil, respectively, 30 minutes after occlusion of right common carotid artery. Right cerebral hemisphere is shown on left of each autoradiogram. Note heterogeneity of reduction of focal cerebral blood flow in moderately symptomatic gerbil in the cerebral cortex, hippocampus, caudoputamen, and thalamus.
within 10 minutes. It is unlikely that a brief temporary occlusion per se affected the clinical outcome in our investigation since we did not detect evidence of neuronal damage or astrocytic reaction with the immunohistochemical method.

We also confirmed the severity of cerebral ischemia morphologically by using the immunohistochemical method for detection of ischemic damage. The extent of ischemic damage 30 minutes after CCA occlusion was different among three symptomatic classes. An observation period of >30 minutes may be necessary to detect ischemic damage among mildly symptomatic gerbils. CBF also was markedly reduced in the moderately and severely symptomatic gerbils. CBF has been measured after unilateral carotid occlusion in gerbils. While focal CBF values after unilateral CCA occlusion were variable in symptomatic gerbils if unselected, they were not variable in our investigation once each gerbil was classified according to clinical status. This was particularly true in severely symptomatic gerbils. In moderately symptomatic gerbils, focal CBF in the vulnerable subregions of the hippo-
campus and cerebral cortex were affected more severely than those in the less vulnerable subregions of the thalamus and caudoputamen. In severely symptomatic gerbils, reduction of focal CBF was very severe and no difference was observed between the vulnerable and less vulnerable subregions. However, there were some subregions without immunohistochemical evidence of ischemic damage even in the presence of profound ischemia, suggesting differences in tissue vulnerability. Our investigation thus demonstrated the feasibility of predicting the severity of cerebral ischemia before permanent occlusion of a CCA in gerbils and of determining the severity soon after permanent occlusion. Our method will be useful for investigating early cerebral ischemia and for evaluating the effect of pharmacologic agents.

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References

KEY WORDS: carotid artery diseases • cerebral blood flow • gerbils
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