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.

From the Department of Neurology, Mayo Clinic and Mayo Foundation, Rochester, Minnesota.
Supported by National Institutes of Health, United States Public Health Service Grant NS-06663.
Address for reprints: T. Yanagihara, MD, Department of Neurology, Mayo Clinic, Rochester, MN 55905.
Received July 13, 1987; accepted October 1, 1987.

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 Carotid Artery Occlusion

Each Observation Period in Gerbils With Permanent Common Carotid Artery Occlusion

Maximal total

Contralateral hemiparesis 1 1 1
Contralateral ptosis 1 1 1
Ipsilateral circling 1 1 1
Disturbance of consciousness 1 2 7
Seizure 2 2 2
Maximal total 7 7 7

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-p,m brain sections corresponding to the stereotaxic section 0.1–0.3 mm rostral and 1.4–1.6 mm caudal to the bregma by using the peroxidase-antiperoxidase method. The antisemur 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...
was examined immunohistochemically. Since the immuno-
histochemical 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,7 the immuno-
histochemical reactions for tubulin and GFAP were
used for these four gerbils.

**Measurement of Focal Cerebral Blood Flow**

The quantitative autoradiographic technique de-
scribed by Sakurada et al.8 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 hydrochlo-
ride, 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.9 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 nCi [14C]iodo-
antipyrene (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.8 At the end of the infusion, the brain
was examined immunohistochemically. Since the im-

---

**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
or appearance 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 reduc-
tion and margin appearance between severely symp-
tomatic 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.

---

**FIGURE 2.** Correlation between diameter (percent size before
temporary ligation) or appearance of common carotid artery
distal to temporary ligation and neurologic outcome after
permanent (30 minutes) occlusion.
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-
TABLE 3. Frequency of Pathologic Lesions in Various Anatomic Regions 30 Minutes After Occlusion of Right Common Carotid Artery in Gerbils

<table>
<thead>
<tr>
<th>Region</th>
<th>Reaction</th>
<th>Severe (n=8)</th>
<th>Moderate (n=9)</th>
<th>Mild (n=5)</th>
<th>Asymptomatic (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>TB</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>100</td>
<td>89</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CA1</td>
<td>TB</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>63</td>
<td>22</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CA3</td>
<td>TB</td>
<td>100</td>
<td>56</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>TB</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>50</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Thalamus</td>
<td>TB</td>
<td>100</td>
<td>33</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>100</td>
<td>44</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Caudoputamen</td>
<td>TB</td>
<td>100</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>TB</td>
<td>88</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>88</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Habenular nucleus</td>
<td>TB</td>
<td>88</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>HE</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are percent abnormal findings for each class based on number of gerbils shown in parentheses. TB, immunohistochemical reaction for tubulin; HE, hematoxylin and eosin staining.

A moderate reduction (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...
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>52.3±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.

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,10 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,2-14 and the clinical signs of cerebral ischemia have been detected 5 minutes after CCA occlusion.2,13 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

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.\textsuperscript{14-17} While focal CBF values after unilateral CCA occlusion were variable in symptomatic gerbils if unselected,\textsuperscript{19} 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-
Matsumoto et al  Prediction of Stroke in Gerbils 497
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.

Acknowledgments

The authors thank Drs. Louis Sokoloff and Charles Kennedy for their advice on cerebral blood flow measurement.

References


KEY WORDS • carotid artery diseases • cerebral blood flow • gerbils
Prediction of stroke before and after unilateral occlusion of the common carotid artery in gerbils.
M Matsumoto, T Hatakeyama, F Akai, J M Brengman and T Yanagihara

Stroke. 1988;19:490-497
doi: 10.1161/01.STR.19.4.490

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/19/4/490

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/