Neurologic and Neuropathologic Outcome After Middle Cerebral Artery Occlusion in Rats

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Focal cerebral ischemia was produced in 45 rats by occlusion of the left middle cerebral artery. Groups of rats were investigated over a long period after occlusion, that is, from a few hours to 42 days after the production of focal ischemia. Light microscopy showed infarcts in the frontoparietal cortex and the lateral caudoputamen. The ischemic changes closely resembled those found in ischemic infarcts in humans and followed a similar pattern over time.

Measurements of the sizes of the infarct, the ipsilateral (operated) hemisphere, and the contralateral hemisphere from camera lucida drawings revealed that the infarct size changed with time after occlusion. Rats killed during the first 7 days (acute phase) had the largest infarcts; in rats killed thereafter, the infarct size diminished. The size of the ipsilateral hemisphere also changed with time; during the first 7 days after occlusion this hemisphere was swollen and larger than the contralateral hemisphere. We suggest that these acute changes are caused by cerebral edema. After the first 7 days, enlargement of the ipsilateral hemisphere gave way to a significant reduction in the size of both the ipsilateral hemisphere and the infarct. We believe that the major reasons for this shift in size are resorption of fluid together with diminished production of edema and elimination of dead cells by macrophages. We suggest that the amount of tissue loss (i.e., the degree of atrophy and the remaining infarct “scar”) found 21–42 days after occlusion (during the late phase) is a measure of the total amount of tissue that succumbed as a consequence of ischemia. Our method of estimating the impact on the brain by measurements during both the acute and the late phases of ischemia seems to be a new and simple way of studying the influence of various treatments on edema and on final brain tissue damage separately. (Stroke 1989;20:641–645)

Tamura et al described a method for the production of focal cerebral ischemia and infarcts in rats based on occlusion of the middle cerebral artery (MCA). This model has attracted much attention because the infarcts in many ways resemble those seen in humans. Further, MCA occlusion in rats causes neurologic deficits that can readily be assessed. The mortality rate is low, and owing to the low cost of laboratory rats it is possible to investigate many animals under various experimental conditions. Further, much comparative data are available concerning diverse aspects of the rat nervous system under experimental conditions causing global ischemia. Apart from that by Nedergaard, most studies of MCA occlusion have concentrated on the acute phase of ischemia, up to a few days after occlusion. However, in a focal ischemic insult the pathophysiological events may continue for longer than a few days. In studies of cerebral edema in MCA-occluded rats using magnetic resonance imaging (K.-Å. Thomus, Z. Kotwica, K. Bergström, L. Persson, H. Bolander, L. Hillered, Y. Olsson, and U. Pontén, unpublished data), we found the accumulation of edema in the ischemic lesion to continue and to change character for more than a week after occlusion. The ischemic lesion appears to be expansive during the first week, raised intracranial pressure having been found in this model (unpublished data). Further, the release from injured nerve cells of a neurospecific protein, neuron-specific enolase, to the cerebrospinal fluid (CSF) took place for approximately 5 days after MCA occlusion,
indicating that neuronal damage may still occur several days after the insult. 13,14

The aim of our present investigation was to study the clinical course and the extent and character of the brain changes in MCA-occluded rats over a long period after occlusion, that is, from a few hours to several weeks after the production of focal ischemia. We believe that it is essential for our future studies on the treatment of focal brain ischemia in this model to know the natural history of infarct development over such a long period of time.

Materials and Methods

We used 45 male Sprague-Dawley rats weighing 370–400 g allowed water and food ad libitum. During the surgical procedure and the perfusion with fixative the rats were anesthetized with an intraperitoneal injection of 1.3–1.5 ml of a mixture containing 4.25% chloral hydrate and 0.97% pentobarbital and were breathing spontaneously.

The MCA was occluded as described by Tamura et al.1 Care was taken to coagulate the MCA from a point proximal to the olfactory tract, including the lenticulostriate artery, to the inferior cerebral vein.3

In preliminary experiments the anesthetic technique was evaluated by monitoring vital features such as arterial blood pressure and blood gases during surgery on the MCA. Neither arterial blood pressure nor blood gases were altered during or immediately after the operation. In rats that were allowed to survive for several days after the occlusion, neurologic examination was performed approximately 24 hours after surgery and again before killing. A neurologic grading system described by Bederson et al 3 and modified by Germano et al 3 was used (Grade 0, normal behavior; Grade I, forelimb flexion; Grade II, forelimb flexion and decreased resistance to lateral push). We found this system useful because the neurologic signs were easy to assess and the grading was reproducible when repeated by different investigators.

Three rats were killed within 8 hours, eight rats between 18 hours and 2 days, seven rats after 3–4 days, eight rats after 5–6 days, nine rats after 7–10 days, seven rats after 21–28 days, and three rats 42 days after the MCA occlusion (Table 1). The rats were anesthetized and perfused through the heart with 200 ml of a 4% buffered formaldehyde solution. The brain was left in situ and immersed in fixative overnight. Three coronal sections of the frontal brain were cut at defined levels (at the level of the bregma, 2 mm in front of the bregma, and 2 mm behind the bregma), embedded in paraffin, and stained with hematoxylin and eosin and van Gieson’s stain. The sections were examined without prior knowledge of time of killing.

Camera lucida drawings were made of the section at the level of the bregma, where the infarct showed its greatest extent, in all brains. From each drawing, the area of the ischemic infarct, the area of the ipsilateral hemisphere (the side of the infarct), and the area of the contralateral hemisphere were calculated with the aid of a computer-based digitizing system (ABC 80, Luxor AB, Motala, Sweden) and expressed as arbitrary area units. The infarct area was expressed as a percentage of the contralateral hemisphere area, considered to be basically unaffected by the ischemic insult, because the area of the entire histologic section varied slightly between brains, probably owing to variations in histologic preparation and magnification of the camera lucida drawings.

For statistical analysis, commercial software (STATVIEW 512+, Brain Power Inc.) on a personal computer (Macintosh, Apple Computer Inc.) was used. A probability level of p<0.05 was considered to be significant.

Results

Table 1 shows the neurologic grades 24 hours after occlusion and at the time of killing. Approximately 10% of the rats were Grade I and 90% were Grade II at 24 hours; 32% of the rats that were killed at 24 hours had Grade II improved to Grade I during the study. Histologically, the ischemic changes closely resembled those found in ischemic infarcts in humans and followed a similar pattern over time. The ischemic lesions were confined to the ipsilateral frontoparietal cortex and to the lateral part of the caudoputamen. The changes were divided into four patterns that developed after MCA occlusion. Overlap occurred, and the stated intervals should be considered as arbitrary.

The earliest detectable changes (1–8 hours after MCA occlusion) affected scattered neurons within the frontoparietal cortex and the lateral part of the caudoputamen. The cell bodies appeared shrunken, had a polygonal form, and were eosinophilic. Such abnormal cells had pyknotic nuclei. The neuropil stained paler and showed areas of microvacuolization and perivascular swelling. During this early phase the ischemic lesion was not clearly demarcated, and sections from rats killed 1–8 hours after MCA occlusion were therefore not used for measuring the infarct area.

Table 1. Experimental Design and Neurologic Grade in Rats 24 Hours After Middle Cerebral Artery Occlusion and Before Killing

<table>
<thead>
<tr>
<th>Survival</th>
<th>At 24 hr</th>
<th>At killing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td>1–8 hr</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18 hr–2 days</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>3–4 days</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>5–6 days</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>7–10 days</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>21–28 days</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>42 days</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are number of rats.
As the infarct matured (18 hours to 3 days after MCA occlusion), coagulation necrosis in the cortex and caudoputamen developed. The border between the infarct and the surrounding tissue was sharp. Remnants of cells in various stages of cytolysis, "cell shadows," were observed in the necrotic tissue. Neutrophil infiltration and a few macrophages were present, particularly in the border zone of the infarct.

Infiltration of macrophages within and at the border of the infarct was a constant finding as the infarcts resolved (4-10 days after MCA occlusion). The infarct was sharply demarcated.

During the late phase (21-42 days) after MCA occlusion, the infarct was still sharply demarcated and surrounded by unaffected cerebral tissue. At 21-28 days a few macrophages were still present, but at 42 days practically none could be found. Dramatic neovascularization had occurred in the infarcted part of the brain, the tissue consisting of vascular trabeculae and some astrocytes. Regions of incomplete infarction were occasionally seen in the caudoputamen of some rats, with a loss of neurons and gliosis. The neuropil appeared spongyform in such incomplete infarcts.

The relative area of the infarct changed significantly with time after MCA occlusion. Linear regression analysis of all rats showed $r = -0.57$ ($p < 0.001$). A significant change over time was also found when Grade I and Grade II rats were analyzed separately ($r = -0.67$ and $r = -0.48$, respectively; $p < 0.05$). Rats killed <7 days after occlusion (during the acute phase) had the largest infarcts; the infarct area diminished in rats killed later (Figure 1). The largest infarcts were seen in the 25 Grade II rats (mean 37%), whereas the 16 Grade I rats and the 12 Grade II rats that improved had smaller (mean 18%) infarcts (Student's $t$ test, $p < 0.001$).

Naked-eye examination of the brain sections from rats killed within the first 7 days showed that the ipsilateral hemisphere was generally larger than the contralateral hemisphere. In contrast, the ipsilateral hemisphere of rats killed 21-42 days after MCA occlusion was strikingly smaller than the contralateral hemisphere (Figure 2). Changes in the size of the ipsilateral hemisphere at various intervals after MCA occlusion are illustrated in Figure 3, together with the values for ipsilateral:contralateral areas; during the first 7 days after MCA occlusion the ipsilateral hemisphere was larger than the contralateral hemisphere. After the first 7 days the ipsilateral hemisphere became smaller, and this diminution became more marked with increasing time after the
MCA occlusion for all rats (linear regression analysis, $r=-0.72$, $p<0.001$). A significant change over time was also found when Grade I and Grade II rats were analyzed separately ($r=-0.65$ and $r=-0.86$, respectively; $p<0.001$).

Because the infarct size and the area of the ipsilateral hemisphere diminished after the first 7 days after MCA occlusion, the area of destroyed cerebral tissue (Figure 2), defined as tissue reduction (contralateral hemisphere area minus ipsilateral hemisphere area) plus the area of remaining infarct “scar,” was calculated and used as an indication of total tissue death caused by ischemia in rats killed during the late phase after MCA occlusion. The mean±SD destroyed cerebral tissue area was 38.4±5.5 area units in six Grade II rats at both 24 hours and at death, whereas it was 24.4±12.2 area units in eight Grade II rats that had been Grade I at 24 hours. This difference in destroyed cerebral tissue area was significant (Mann-Whitney $U$ test, $p<0.01$).

**Discussion**

Our study shows that MCA-occluded rats can survive for several weeks, during which period many will improve neurologically. The neurologic grades 24 hours after MCA occlusion and at death were related to infarct size. Also, the final tissue loss (i.e., destroyed cerebral tissue) of rats allowed to survive >7 days was related to the clinical course: Grade II rats that improved neurologically had less destroyed cerebral tissue than rats with a stable neurologic deficit.

The histologic and naked-eye findings were time-related and resembled those seen in humans, further implying that MCA occlusion in rats is a useful model for studying focal ischemia. The late changes, too, resembled those seen in ischemic infarcts in humans; for example, dilatation of the ipsilateral ventricle was observed.

During the acute phase of ischemia, the ipsilateral hemisphere was enlarged, presumably largely owing to the accumulation of edema fluid, whereas vasodilatation and cell infiltration probably played minor roles. We have recently observed that intracranial pressure is raised (unpublished data) and that cerebral edema can be detected by magnetic resonance imaging (K-Å. Thomas, Z. Kotwica, K. Bergström, L. Persson, H. Bolander, L. Hillered, Y. Olsson, and U. Pontén, unpublished data) in rats investigated within approximately 7 days after MCA occlusion. After the first week, the enlargement of the hemisphere gives way to a significant reduction in the size of both the ipsilateral hemisphere and the infarct. We believe that the major reasons for this shift in size are resorption of fluid together with diminished production of edema and the elimination of dead cells by macrophages.

During the acute phase of focal ischemia, the size of the ischemic lesion as an area measured on a histologic section appears to depend on two closely related phenomena, the amount of necrotic tissue and the amount of cerebral edema. During the late phase, the amount of destroyed cerebral tissue calculated from a histologic section is probably largely an expression of the total number of cells that died as a result of ischemia. We used the area of destroyed cerebral tissue as a measure of cell death because it takes into account both the area of reduction of the hemisphere (i.e., atrophy) and the area of the tissue lost but replaced by vascular trabeculae (i.e., the infarct “scar”).

The effect on the sizes of the ipsilateral hemisphere and the infarct during the acute phase on the one hand and the degree of destroyed cerebral tissue during the late phase on the other hand appear to be closely related because they both depend on the severity of the ischemic insult. However, a prerequisite for this relation seems to be that their measurements be made on MCA-occluded rats from the same population during both the phase of maximal expansion and the phase of maximal hemisphere reduction. The introduction of various experimental procedures, such as therapeutic maneuvers, may alter the relation between the degree of expansion during the acute phase and the degree of tissue loss during the late phase simply because such maneuvers may predominantly influence either edema or cell injury. Our method of estimating the impact on the brain by measuring area during the late phase also seems to be a new and simple way of studying the influence of various treatments instituted at various times before or after MCA occlusion in rats. Thus, by comparing the effects of therapy on infarct size and hemisphere expansion during the acute phase and the effects on destroyed cerebral tissue area during the late phase, it appears possible to differentiate between effects on cerebral edema and on cell injury. Treatment against the latter has been called “cerebral protection” and infers that ischemic tissue injury can be reduced or prevented. Therapeutic maneuvers instituted with this object in our model ought to reduce the degree of destroyed cerebral tissue during the late phase after ischemia, with or without having an effect on infarct size or hemisphere expansion during the acute phase. On the other hand, agents with antiedema properties ought to reduce hemisphere expansion and perhaps infarct size during the acute phase but not necessarily affect the final destroyed cerebral tissue area.

Our study thus underlines the importance of time for interpreting ischemic changes, and our results suggest that for evaluating various therapies observations should be made during both the acute and late phases of focal ischemia. Edema formation should be evaluated during the acute phase of ischemia; if combined with observations during the late phase, effects on edema and tissue injury could then be studied separately. The possibility of investigating separately and quantitatively possible effects on cerebral edema and on tissue injury has obvious clinical implications. Certain drugs and therapies.
have been claimed to offer cerebral protection, that is, to prevent or reduce cerebral tissue injury. In clinical practice it is often difficult to evaluate effects on tissue injury because this cannot be directly measured in patients. Instead, tissue injury is often appraised on the basis of clinical status and outcome; such assessment is difficult in patients with severe brain damage because they constitute a heterogeneous group and because numerous other factors confound the picture. Further, therapeutic measures aimed at reducing tissue injury may at the same time exacerbate cerebral edema. For example, induced arterial hypertension and hypervolemia improve cerebral blood flow in the ischemic area and may moderate ischemic tissue injury; however, although effective in terms of rapid improvement of neurologic status, such treatment may aggravate the formation of ischemic edema, which in turn may result in increased intracranial pressure and brain herniation, countering any beneficial effect on tissue injury. Therefore, the relation between the degree of cerebral edema and tissue injury can be altered by this kind of treatment. Although the goal of treatment is to improve clinical outcome, cerebral protection is used to limit the extent of tissue injury, and methods allowing direct evaluation of this particular effect are clearly important. Our experimental design appears to be useful in this respect.

Methods for the direct estimation of ischemic cell injury in patients are also urgently required. In an attempt to approach this problem from another angle, we studied the release from injured cerebral tissue to the CSF of two neurospecific proteins, neuron-specific enolase and S-100 protein, in patients with ischemic brain damage. The findings gathered so far indicate a relation between the CSF concentrations of these proteins and the degree of tissue injury. Because it is impossible to measure directly the cerebral tissue injury, we adopted the MCA occlusion model for more detailed studies on the relation between the release of these two proteins to CSF and the actual cell injury. We plan to extend these studies using our current experimental design.

The focal ischemic changes produced by MCA occlusion constitute a dynamic process in which several pathophysiological events occur over an extended period. Our model promises to be useful for the evaluation of various anti-ischemic therapies. We suggest that in such studies the observation time should be not less than 42 days after occlusion to allow estimation of the final tissue injury caused by the ischemia.

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References


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