Penumbral Tissues Salvaged by Reperfusion Following Middle Cerebral Artery Occlusion in Rats

Hajime Memezawa, MD; Maj-Lis Smith, PhD; and Bo K. Siesjo, MD, PhD

Background and Purpose: The rat is now extensively used for studies on focal cerebral ischemia, and several novel pharmacological principles have been worked out in rat models of middle cerebral artery occlusion. The objective of the present study was to assess how ischemic tissue can be salvaged by reperfusion in a model of transient focal ischemia that gives infarction of both the caudoputamen and the neocortex.

Methods: The middle cerebral artery of anesthetized rats was occluded for 15, 30, 60, 90, 120, or 180 minutes by an intraluminal filament, and recirculation was instituted for 7 days to allow assessment of the density and localization of ischemic brain damage using histopathologic techniques. Local cerebral blood flow was measured in separate animals to verify that removal of the filament was followed by adequate recirculation.

Results: Following 15 minutes of middle cerebral artery occlusion seven of eight rats showed selective neuronal necrosis in the caudoputamen, while the neocortex was normal. After 30 minutes of occlusion, seven of eight animals had infarcts localized to the lateral caudoputamen, and four of eight had selective neuronal necrosis in the neocortex. Prolongation of the ischemia to 60 minutes induced cortical infarction in all eight rats. The infarct size increased progressively with increasing occlusion time, up to 120–180 minutes, when the infarcts were as extensive as those observed following 24 hours of permanent middle cerebral artery occlusion.

Conclusions: The results demonstrate a time window for salvage of penumbral tissues by reperfusion that is shorter than that suggested on the basis of previous data in other species. The results probably reflect a lower collateral blood flow in the rat than in other species. This should be taken into account when the effect of pharmacological agents is studied in rats. (Stroke 1992;23:552–559)

KEY WORDS • cerebral infarction • cerebral ischemia • reperfusion • rats

It is now widely believed that a stroke lesion usually consists of a densely ischemic core and perifocal areas (the penumbra) that subsequently may become recruited in the infarction process. Penumbral areas were originally defined as those having a reduction in cerebral blood flow (CBF) sufficiently severe to extinguish spontaneous or evoked electrical potentials, yet sufficiently mild to allow maintenance of membrane potentials and gross cellular ion homeostasis. It seems advantageous to adopt a wider definition of the ischemic penumbra and let it denote ischemic areas that can be salvaged by pharmacological agents or by relatively prompt reperfusion. So far, most available information concerns penumbral tissues in permanent middle cerebral artery (MCA) occlusion. This is because competitive and noncompetitive N-methyl-D-aspartate (NMDA) antagonists have been shown to reduce infarct size to about 50% of control even though MCA occlusion is maintained. Comparable effects have been obtained with some calcium antagonists. In all these cases, pharmacological treatment reduced damage to neocortical tissue without affecting the lesion to the focal areas encompassing the dorsolateral part of the caudoputamen and the adjacent neocortical areas.

Less is known about salvage of perifocal tissues by reperfusion. Sundt et al found that infarcts could be avoided if reperfusion was instituted within 2–3 hours in cats and 3–6 hours in monkeys. More recently, Weinstein et al reported extensive data in cats. Animals with up to 3 hours of MCA occlusion showed good recovery, and brain sections showed only scattered necrotic areas. Similar studies have been carried out in monkeys. In this species, infarction was observed only if local CBF was reduced below 0.10–0.12 ml·g⁻¹·min⁻¹ for 2–3 hours. The results showed that 15–18 minutes of MCA occlusion was tolerated without histological lesions, while an occlusion of 30 minutes gave either no damage or microscopic lesions confimed

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TABLE 1. Local Cerebral Blood Flow in Different Brain Structures After 15 Minutes of Recirculation in Rats Subjected to 60 Minutes of Transient Unilateral Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Structure</th>
<th>Occluded hemisphere</th>
<th>Contralateral hemisphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior cingulate cortex</td>
<td>0.81±0.23*</td>
<td>2.18±1.25</td>
</tr>
<tr>
<td>Frontoparietal cortex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Motor area</td>
<td>0.41±0.24†</td>
<td>2.18±0.63</td>
</tr>
<tr>
<td>Upper somatosensory area</td>
<td>0.68±0.48†</td>
<td>2.14±0.60</td>
</tr>
<tr>
<td>Lower somatosensory area</td>
<td>1.16±0.95</td>
<td>2.39±1.05</td>
</tr>
<tr>
<td>Caudoputamen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral</td>
<td>0.96±0.56‡</td>
<td>2.12±0.54</td>
</tr>
<tr>
<td>Medial</td>
<td>1.06±0.63</td>
<td>1.53±0.27</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>1.00±0.52</td>
<td>1.12±0.35</td>
</tr>
<tr>
<td>Thalamus</td>
<td>1.30±0.23†</td>
<td>2.71±0.52</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>0.90±0.71‡</td>
<td>2.61±0.78</td>
</tr>
<tr>
<td>Piriform cortex</td>
<td>0.55±0.30‡</td>
<td>1.88±0.82</td>
</tr>
<tr>
<td>Amygdala</td>
<td>0.57±0.27‡</td>
<td>1.63±0.70</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.42±0.17‡</td>
<td>1.01±0.30</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>2.07±0.78</td>
<td>1.62±0.30</td>
</tr>
<tr>
<td>Auditory cortex</td>
<td>0.61±0.36†</td>
<td>2.80±1.04</td>
</tr>
</tbody>
</table>

Values are mean±SD ml·g⁻¹·min⁻¹ for six rats. *p<0.05, †p<0.001, and ‡p<0.01 different from contralateral value by Scheffe's F test.

Materials and Methods

Male Wistar rats (Mellegaard Breeding Center, Copenhagen, Denmark) weighing 290–350 g were used.
TABLE 2. Physiological Parameters of Rats in Histopathologic Study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Period of occlusion (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 (n=8)</td>
</tr>
<tr>
<td>Rectal temperature (°C)</td>
<td>37.0±0.3</td>
</tr>
<tr>
<td>Scalp temperature (°C)</td>
<td>36.8±0.4</td>
</tr>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>119±13</td>
</tr>
<tr>
<td>PCO₂ (mm Hg)</td>
<td>39.6±3.5</td>
</tr>
<tr>
<td>PO₂ (mm Hg)</td>
<td>120±11</td>
</tr>
<tr>
<td>pH</td>
<td>7.41±0.02</td>
</tr>
<tr>
<td>Blood glucose (mM)</td>
<td>6.4±0.8</td>
</tr>
</tbody>
</table>

During occlusion

| Rectal temperature (°C)    | 37.2±0.2 | 37.1±0.2 | 37.1±0.3 | 36.9±0.1 | 36.9±0.4 | 36.8±0.1 |
| Scalp temperature (°C)     | 36.7±0.1 | 36.9±0.2 | 36.7±0.1 | 36.9±0.3 | 37.1±0.3 | 36.7±0.3 |
| Blood pressure (mm Hg)     | 130±12   | 120±16   | 122±17   | 110±14   | 104.0±5.3 | 113±2     |
| PCO₂ (mm Hg)               | 37.8±1.9 | 38.4±2.6 | 40.7±2.8 | 41.4±4.0 | 40.8±2.9 | 38.3±3.5 |
| PO₂ (mm Hg)                | 119±9    | 126±11   | 125±8    | 123±13   | 123.1±20.2 | 114±11    |
| pH                         | 7.43±0.01 | 7.45±0.05 | 7.42±0.02 | 7.41±0.04 | 7.4±0.0  | 7.43±0.01 |
| Blood glucose (mM)         | 6.2±0.8  | 6.1±0.5  | 6.0±0.6  | 6.3±0.7  | 6.3±0.7  | 5.9±1.0   |

Values are mean±SD. Measurements before occlusion were made 5–10 minutes before insertion of occluder filament in middle cerebral artery. Measurements during occlusion were made 5–10 minutes after occlusion.

Consisted of two pieces: an occluder filament (made from a nylon monofilament thread 0.28 mm in diameter sold as 0.25 mm by Platil, F.R.G.) and a guide sheath (made from a polyethylene catheter [PE-10], Portex Co., Kent, England). The right common, internal, and external carotid arteries were identified through a cervical midline incision. The external carotid artery and the occipital artery (one of the branches of the external carotid artery) were ligated by a 3-0 silk suture. The pterygopalatine artery, which is a branch of the internal carotid artery) were ligated by a 3-0 silk suture. The pterygopalatine artery, which is a branch of the internal carotid artery, was encircled with a suture and retracted from a nylon monofilament thread 0.28 mm in diameter flush with saline, the animals were perfusion-fixed with 1.0% halothane in N₂O/O₂ (70%: 30%). A thoracotomy was made, and a cannula was inserted into the ascending aorta via the left ventricle. After a short flush with saline, the animals were perfusion-fixed with phosphate-buffered 4% formaldehyde (pH 7.4). Then the brains were removed from the craniums, dehydrated, embedded in paraffin, sectioned coronally at 5 μm, and stained with a combination of celestine blue and acid fuschin. In the histopathologic examination, outlines of the infarct were drawn on brain maps and the localization of selective neuronal necrosis was noted.

Local CBF was studied autoradiographically in six rats subjected to 60 minutes of MCA occlusion followed by 15 minutes of recirculation. Four sham-operated animals had the occluding device inserted into the internal carotid artery, but the nylon filament was not advanced to occlude the MCA. Local CBF was measured in the awake, minimally restrained rats with the technique of Sakurada et al²⁴ as modified by Dahlgren et al.²⁵ Carbon-14-labeled iodoantipyrine (Amersham) was used as the radioactive tracer. Isotope infusion time was 30 seconds, during which arterial blood was sampled at 3-second intervals. The animals were decapitated simultaneous with the last arterial sample, and the brains were rapidly frozen and later processed for autoradiography. Local CBF was evaluated as described by Sakurada et al²⁴ with the help of an IBAS image analyzer (Kontron Bildanalyse GmbH, Munich, F.R.G.).²⁶

Statistical analyses were performed using analysis of variance, followed by Scheffé's F test; p<0.05 was regarded as significant. Values are presented as mean±SD.
Results

Ten rats were used for measurements of local CBF. Values of the physiological variables (not shown) were normal and did not differ from those of the histopathology study (see below). The four sham-operated animals showed a slightly lower CBF on the sham-occluded side (which had the internal carotid artery occluded) than on the contralateral side. This side difference was, however, not significant in any measured structure. The mean±SD value for all structures on the sham-occluded side as a percent of that of the contralateral structures was 84±7%. Recirculation local CBF was studied in six rats following 60 minutes of unilateral MCA occlusion. The local CBF values are given in Table 1. Fifteen minutes after withdrawal of the occluding filament all animals showed reperfusion in the previously ischemic structures but no signs of hyperemia (Figure 1). On the contrary, local CBF in these structures ranged from 19% (frontoparietal cortex, motor area) to 47% (lateral caudoputamen) of the contralateral value. Of the two mentioned structures, the lateral caudoputamen suffers the more pronounced ischemia during MCA occlusion. Local CBF values in the substantia nigra were bilaterally somewhat higher in the postischemic state than in the sham-operated rats, but the increase did not reach the level of significance.

Fifty-one animals were subjected to transient MCA occlusion followed by recovery for 7 days before fixation for histopathologic examination. The rats' neurological deficits were checked 15–20 minutes after vascular occlusion. All animals except three showed gait disturbances (circling to the left or gait to the left), and the three asymptomatic rats were excluded from the experiment. All animals that suffered 15, 30, or 60 minutes of MCA occlusion (n=8 each) survived for 7 days, but of those subjected to 90 (n=9), 120 (n=8), or 180 (n=7) minutes of MCA occlusion, one, five, and four rats, respectively, died during the first 2 postinsult days with signs of massive brain edema.
Table 2 shows the physiological parameters in these animals before and during MCA occlusion. The rats were normothermic, normotensive, and normocapnic and had normal blood glucose concentrations and arterial pH. The blood pressure fell transiently by 10–15 mm Hg during 2–3 minutes immediately after MCA occlusion (not in the table) but rose to stable values when isoflurane anesthesia was discontinued.

Figure 2 gives an overview of the histopathologic changes after 7 days of reperfusion following 15, 30, 60, 90, 120, or 180 minutes of MCA occlusion. Fifteen minutes of occlusion resulted in selective neuronal necrosis confined to the caudoputamen in seven of eight animals; one rat showed no brain damage. The eight animals with 30 minutes of occlusion had infarcts localized to the lateral caudoputamen in seven, and four had selective neuronal necrosis in the neocortex. Figure 3 illustrates the histological appearance in one rat of infarction in the lateral caudoputamen (Figure 3A) and of selective neuronal vulnerability in the neocortex (Figure 3B). Prolongation of MCA occlusion to 60 or 90 minutes led to extension of the infarcts to the neocortex in eight of eight and seven of eight animals, respectively. When cortical infarction appeared, selective neuronal vulnerability was often seen close to the infarct, extending into the superficial and deep cortical layers (Figure 4). In all rats subjected to 120 or 180 minutes of MCA occlusion, the infarcted region extended to the whole caudoputamen and most of the ipsilateral cortex excluding the cingulate cortex, which is supplied with blood from the anterior cerebral artery. We measured the infarcted area in the different groups at the levels of the caudoputamen, hippocampus, and substantia nigra and, as Figure 5 demonstrates, the infarct size increased progressively with increasing MCA occlusion time.

**Discussion**

If the ischemic penumbra is defined as the perifocal tissues that can be salvaged by either pharmacological treatment or relatively prompt reperfusion, it becomes of considerable interest to assess metabolic and circulatory events in focal and perifocal areas. As stated in the introduction, pretreatment of animals with certain drugs, including NMDA antagonists, seems to reduce the size of the infarct resulting from permanent MCA occlusion to about 50% of control. The tissue salvaged, encompassing large parts of the neocortex, would then constitute the penumbra. Obviously, the reduction in blood flow and the perturbation of metabolism are less pronounced in these perifocal than in focal tissues, allowing the tissue to be preserved if it is protected from the harmful effects of moderate ischemia during the initial, critical phase.

In two previous articles, we have assessed the size of the infarct that results from 1 hour of transient MCA occlusion or permanent MCA occlusion, the focal and perifocal blood flow rates, and the accompanying changes in labile tissue metabolites. The present article concerns ischemic tissues that can be salvaged by reperfusion following occlusion periods varying between 15 and 180 minutes. The questions raised were
first, how quickly must reperfusion be achieved to salvage focal tissues in the caudoputamen and neocortex and second, what is the reperfusion time that will salvage penumbral tissues (i.e., those protected by drugs in a setting of permanent MCA occlusion)?

As mentioned in the introduction, many previous attempts have been made to assess the effect of recirculation on the size of the resulting infarct. Results obtained in both cats and monkeys are consistent with a gradual development of selective neuronal necrosis and of infarction during the first 4–8 hours. However, the variability was relatively large, and it is difficult to define accurately the occlusion periods that will not lead to any damage or those that can be tolerated without the development of infarction. Part of this variability must reflect interanimal differences in collateral blood supply and another part must reflect species differences.

Since the pharmacology of cerebral ischemia is to a large extent worked out in rats, and based on the models described by Tamura et al.\textsuperscript{19} and others, it is highly justified that salvage of tissues by reperfusion is worked out in the same species. At present, the most extensive reperfusion data have been reported with the model that is based on a distal MCA occlusion combined with common carotid artery occlusion.\textsuperscript{28,29} For example, Buchan et al.\textsuperscript{30} have studied pharmacological amelioration of damage due to transient occlusion and Kaplan et al.\textsuperscript{18} have studied the reperfusion “window.” The latter
authors reported results that are similar to those previously reported for cats and monkeys, namely, that 1 hour of occlusion gives no or only a small infarct. However, their maximal reperfusion window (about 3 hours) is shorter than that reported for the larger species, although the reduction in blood flow was relatively moderate. The intraluminal filament model of Koizumi et al\textsuperscript{20} seems ideally suited to study tissues salvaged by reperfusion, and it has the advantages of affecting the caudoputamen as well. No such study has hitherto been made, but perifocal blood flow rates suggest that some neocortical areas could represent penumbral tissues.\textsuperscript{21,22} Abe et al\textsuperscript{23} reported that although local CBF during occlusion was similar in the lateral caudoputamen and neocortex, the caudoputamen showed inhibition of protein synthesis (and formation of edema) that started earlier and resolved later than in the neocortex. In that study, 1 hour of ischemia did not seem to yield irreversible tissue damage in either region. Longer periods of ischemia were studied by Nagasawa and Kogure,\textsuperscript{21} but since reperfusion was not instituted or was brief, infarction could not be studied. Our own previous results showed that reperfusion following 1 hour of ischemia gave a smaller infarct than 24 hours of permanent occlusion; however, shorter or longer ischemic periods were not studied.\textsuperscript{22}

In view of previous reports, our present results were unexpected. Thus, seven of eight rats with 30 minutes of occlusion had infarcts in the lateral part of the caudoputamen, and four of eight had clear neocortical damage (two additional animals had damage localized to the border zone between the anterior and middle cerebral arteries and to the entorhinal cortex, respectively). The damage observed after 1 hour of ischemia was also relatively extensive. Thus, although the damage was less than after 120–180 minutes of occlusion, it nonetheless encompassed infarcts in the caudoputamen and neocortex in seven of eight rats. Finally, our results demonstrate that although reperfusion after 90 minutes was beneficial, reperfusion after 2 hours of ischemia failed to salvage tissue. This follows from the finding that infarct size was similar after 120 and 180 minutes of occlusion and that mortality was at least as high in the 120 minute group as in the 180 minute group. Our results thus demonstrate more extensive damage after 30 minutes and 1 hour of occlusion than that reported by Kaplan et al,\textsuperscript{18} and the reperfusion window for salvage of some tissue was clearly shorter (1.5–2 hours compared with 3–4 hours).

The question arises of why there is brain damage incurred in reversible MCA occlusion of 30 minutes' duration. It is perhaps predictable that the caudoputamen should be damaged by this duration of ischemia, particularly since blood flow in the caudoputamen is reduced to 5–10% of control.\textsuperscript{22} This is a flow rate that is similar to or only slightly higher than that observed in forebrain ischemia in rats.\textsuperscript{30,32,33} We recall that with these models, ischemia of 15–30 minutes' duration invariably gives extensive damage to the caudoputamen.\textsuperscript{32,34} It remains to be discussed, therefore, why some previous studies on larger animals have failed to show infarction in the caudoputamen after much longer periods of ischemia. In all probability, the discrepancy can be explained by variations in collateral blood flow between species and between models. A likely contributing factor is temperature, which may differ among investigations.\textsuperscript{34} It is, however, of importance to note that the cortex, suffering a similar reduction of blood flow at a similar temperature as the caudoputamen during MCA occlusion,\textsuperscript{22} is more resistant to tissue damage, suggesting a selective vulnerability in the caudoputamen of still unknown origin (see Reference 31). The time window for salvage of a major part of the neocortex demonstrated in the present results is maximally 60 minutes, and the window for some parts of the neocortex is 90–120 minutes. Again, this is a shorter period than that expected from most previous work. The reasons are not obvious, but the results may reflect the relatively dense ischemia in rats and a relatively marked metabolic perturbation in species of that size. However, although the results of Kaplan et al\textsuperscript{18} attest to that fact, their results, which pertain to infarction restricted to the neocortex, demonstrate a somewhat slower evolution of cortical infarction. In all probability, this can be explained by the higher blood flow rates obtained in the focus of their ischemic lesion. It is clearly justified that such model- and species-related differences in the rate of evolution of an infarct are taken into account when the results of pharmacological interventions are evaluated.

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