What Is Effective in Malignant Middle Cerebral Artery Infarction: Reperfusion, Cranectomy, or Both? An Experimental Study in Rats

T. Engelhorn, MD; R. von Kummer, MD; W. Reith, MD; M. Forsting, MD; A. Doerfler, MD

Background and Purpose—We sought to evaluate the effects of reperfusion and cranectomy treatment at different time points after middle cerebral artery (MCA) occlusion on infarct volume and neurological outcome in MCA infarction in rats.

Methods—We used an endovascular technique to obtain MCA occlusion in 182 rats. Thirteen groups with 14 animals each were investigated: control group 1 with no treatment; groups 2 to 7 with only reperfusion or cranectomy at 1, 4, or 12 hours, respectively; and groups 8 to 13 with reperfusion at 1 or 4 hours combined with cranectomy at 1, 4, or 12 hours, respectively. We used infarct volume and neurological performance as study end points in all animals at day 7.

Results—Neurological score and infarct volume in animals undergoing early reperfusion at 1 hour were significantly smaller than those in control animals (3.8/225 ± 26 mm³) (P < 0.01). Reperfusion at 4 hours (2.8/182 ± 62 mm³) and 12 hours (3.7/231 ± 69 mm³) did not result in significant improvement. Animals undergoing cranectomy at 1, 4, and 12 hours demonstrated significantly better outcome and significantly reduced infarct volume (1.6/96 ± 30 mm³, 1.9/109 ± 39 mm³, and 2.6/150 ± 34 mm³, respectively) (P < 0.05). Compared with 1 treatment at a time, combined reperfusion and cranectomy did not result in a significant additional benefit.

Conclusions—Early reperfusion and cranectomy at 1 hour are both effective in large MCA infarction. While reperfusion later than 1 hour was not beneficial, late cranectomy at 4 and 12 hours still resulted in significant improvement of neurological score and reduction of infarction size. Combined treatment at different time points yields no significant additional benefit compared with 1 treatment at a time. (Stroke. 2002;33:617-622.)

Key Words: brain ischemia  cerebral infarction  cranectomy  reperfusion  rats

Oclusion of the internal carotid artery (ICA) or middle cerebral artery (MCA) (segment M1) occurs in 10% to 15% of stroke patients and may lead to massive cerebral edema with raised intracranial pressure (ICP) and progression to coma and death. Mortality rates are reported to be up to 80% in this type of cerebral infarction, leading to the term malignant MCA infarction. With the emergence of aggressive therapies for ischemic stroke, such as thrombolysis and cranectomy, clinical prognosis of these infarcts could potentially be improved. The therapeutic time window for beneficial treatment is of particular importance. For maximal benefit, both reperfusion and cranectomy must be established early after onset. In the clinical setting, the beneficial time window for thrombolysis has been shown to be approximately 3 hours. In the rat model of endovascular MCA occlusion, the time window for beneficial reperfusion is reported to be approximately 2 hours. Reperfusion later than 2 hours may accelerate postischemic brain edema, leading to “reperfusion injury” probably mediated by different mechanisms, such as an increase in blood-brain barrier permeability, hemorrhagic transformation, release of excitatory amino acids, expression of endothelial adhesion molecules, elevation of prostaglandins, and apoptosis.

For hemispheric MCA infarction, cranectomy has shown to be effective in experimental studies and for selected patients in clinical studies, indicating maximal benefit if performed early after symptom onset. In a study in rats, cranectomy significantly reduced mortality and improved clinical outcome in a time-dependent fashion even if performed 12 hours after MCA occlusion.

Theoretically, combined treatment of reperfusion and cranectomy might be beneficial because a major disadvantage of reperfusion—reperfusion injury with exacerbation of brain edema and elevation of ICP—could be counterbalanced by cranectomy, thereby potentially prolonging the time window for a beneficial reperfusion. Therefore, the present study was designed to evaluate the singular and combined effects of cranectomy and/or reperfusion at different time points after middle cerebral artery (MCA) occlusion on infarct volume and neurological outcome in MCA infarction in rats.

Results—Neurological score and infarct volume in animals undergoing early reperfusion at 1 hour were significantly smaller than those in control animals (3.8/225 ± 26 mm³) (P < 0.01). Reperfusion at 4 hours (2.8/182 ± 62 mm³) and 12 hours (3.7/231 ± 69 mm³) did not result in significant improvement. Animals undergoing cranectomy at 1, 4, and 12 hours demonstrated significantly better outcome and significantly reduced infarct volume (1.6/96 ± 30 mm³, 1.9/109 ± 39 mm³, and 2.6/150 ± 34 mm³, respectively) (P < 0.05). Compared with 1 treatment at a time, combined reperfusion and cranectomy did not result in a significant additional benefit.
points after endovascular MCA occlusion in rats on infarct volume and neurological outcome.

## Materials and Methods

### Animal Model and Experimental Protocol

We induced focal cerebral ischemia in 182 male Sprague-Dawley rats (weight, 290 to 320 g) using an intraluminal suture occlusion model of the MCA. The study was approved by the local animal protection committee. Animals were anesthetized with ketamine (4 mg/100 g) and xylazine (1.5 mg/100 g) by intramuscular injection and were allowed free access to food and water before the procedure. Monitoring of hematocrit, pH, Po2, Pco2, and blood pressure during surgery was performed with the use of a femoral artery catheter. Rectal temperature was maintained at 37°C with a feedback-regulated heating pad during the operation.

In all animals, the right MCA was occluded via a transvascular approach, as previously described in detail. Briefly, all branches of the right common carotid artery and external carotid artery (ECA) were isolated and ligated. The ICA was then isolated, and its extracranial branch, the pterygopalatine artery, was ligated close to its origin. A 4-0 silicone-coated nylon suture (Ethilon black monofilament nylon 4-0, Ethicon, Inc) was introduced into the transected ECA and gently advanced into the ICA and circle of Willis to close the origin of the MCA. For reperfusion, the suture was withdrawn back into the ECA to restore ICA-MCA blood flow.

We performed craniectomy by creating a bone flap (10×5 mm) in the parietal and temporal bone using a dental drill. The dura covering the parietal and temporal lobes was then opened by a large cruciate incision. We did not attempt to resect infarcted brain tissue. At the end of the procedure the temporals muscle and skin flap were adapted and sutured in place.

The 182 animals were allocated to 13 treatment groups (n=14 in each group) with the use of a computer randomization scheme (Table 1). Animals of group 1 (controls) remained untreated after MCA occlusion. Forty-two animals underwent reperfusion only at 1 hour (group 2), 4 hours (group 3), or 12 hours (group 4) after MCA occlusion. In 42 animals craniectomy only was performed at 1 hour (group 5), 4 hours (group 6), or 12 hours (group 7) after MCA occlusion. Eighty-four animals underwent combined treatment with reperfusion and craniectomy. Reperfusion at 1 hour after MCA occlusion was followed by craniectomy at 1 hour (group 8), 4 hours (group 9), or 12 hours (group 10) after MCA occlusion. In 3 additional groups, reperfusion at 4 hours after MCA occlusion was combined with craniectomy at 1 hour (group 11), 4 hours (group 12), or 12 hours (group 13) after MCA occlusion.

We anesthetized all animals (including control animals) initially for 4 hours for MCA occlusion and for reperfusion or and decompressive craniectomy at 1 and 4 hours after MCA occlusion. Animals treated with reperfusion or craniectomy at 12 hours after MCA occlusion underwent a second administration of anesthesia for approximately 30 minutes. At day 7, we examined all surviving animals neurologically using an established scoring system first introduced by Bedersen et al and refined by Menzies et al (Table 2) and simultaneously measured the body weight.

### Data Analysis

After digitization of the photographs, we quantified the area of infarction using a public domain software for Macintosh computers (IMAGE J.1, Wayne Rasband, National Institutes of Health). We marked on each slice the unstained area (ischemic brain) and calculated the infarct volume according to the slice thickness of 1 mm per slice. Measurement and calculation of infarction size were done by an investigator blinded to the treatment groups. To avoid overestimation of the infarction volume, as described by Lin et al, the corrected infarction volume (CIV) is given by $CIV = (LT - (RT - RI)) \times d$, where $LT$ is the area of the left hemisphere in mm², $RT$ is the area of the right hemisphere in mm², and $d$ is the thickness of the slices. The ischemic lesion volume was expressed as absolute volume (mm³).

### Statistical Analysis

For statistical analysis of all results, commercial software (StatView, Brain Power Inc) was used. For statistical analysis of infarction volume and body weight, the unpaired t test was used; for statistical analysis of the neurological score, the Kruskal-Wallis test was used.

<table>
<thead>
<tr>
<th>TABLE 2. Neurological Score After MCA Occlusion According to Bedersen et al and Menzies et al</th>
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MCA indicates MCA occlusion.
A probability value of \( P < 0.05 \) was considered significant. Means and SDs are presented for the various groups.

### Results

We excluded a total of 26 animals (14%) from data analysis. Thirteen animals demonstrated respiratory problems, bleeding, or a significant change in physiological parameters during anesthesia and preparation (n = 13). Four animals died within 2 hours after advancement of the suture. These animals demonstrated subarachnoid hemorrhage due to suture perforation. Additionally, 9 animals revealed no infarction because of unsuccessful MCA occlusion.

We noted no statistically significant differences in the remaining 156 animals among the 13 groups for any of the intraoperative physiological parameters. Throughout animal preparation, the mean ± SD body temperature for all animals was 36.9 ± 0.5°C. Arterial blood gases (\( P_{O2} = 98 ± 17 \), \( P_{CO2} = 34 ± 5.2 \), pH = 7.38 ± 0.04) and hematocrit (41.3 ± 2.9) remained stable.

### Mortality

Ten of 156 successfully prepared animals died during anesthesia and preparation (n = 13). Four of 12 animals of the control group died (mortality rate, 33%), and 2 of 13 animals undergoing reperfusion 12 hours after MCA occlusion died (mortality rate, 15%). None of the animals undergoing only craniectomy treatment died. Combined therapy resulted in mortality rates of 17% (2 of 12 animals) in group 8 (reperfusion and craniectomy at 1 hour after MCA occlusion) and 9% (1 of 11 animals) in groups 10 and 13 (reperfusion at 1 and 4 hours, respectively, combined with craniectomy at 12 hours after MCA occlusion).

### Body Weight

All animals of the study had a decline of body weight. Table 3 illustrates the weight changes. Body weight is expressed as percentage of initial weight. Seven days after MCA occlusion, the average body weight of the control group was 71.0 ± 3.3%. Except in animals of groups 3 (reperfusion at 4 hours after MCA occlusion), 4 (reperfusion at 12 hours after MCA occlusion), and 13 (reperfusion at 4 hours combined with craniectomy at 12 hours after MCA occlusion), the weight differences of treated animals were statistically significantly better, i.e., smaller than those of control animals (\( P < 0.05 \)). Although reperfusion at 4 hours after MCA occlusion did not result in a significantly smaller loss in weight (72.2 ± 8.8%), when combined with craniectomy at 1 or 4 hours after MCA occlusion there was a significant improvement (85.0 ± 11.8% and 80.8 ± 4.7%, respectively) (\( P < 0.05 \)).

### Neurological Score

Table 3 and Figure 1 illustrate the neurological score at day 7 after MCA occlusion. The average neurological score of the control group was 3.8 ± 1.2. Except animals of group 4 (reperfusion at 12 hours after MCA occlusion; 3.7 ± 1.0), all treated animals demonstrated a statistically better (ie, lower) neurological score. Animals treated by craniectomy at 1 hour after MCA occlusion achieved a slightly better neurological score at day 7 compared with animals treated by reperfusion at 1 hour after MCA occlusion (1.6 ± 0.5 compared with 1.8 ± 0.6; \( P > 0.31 \)).

### TTC-Derived Infarct Volume

Table 3 and Figure 2 show TTC-derived infarct volume at day 7 after MCA occlusion. Figure 3 illustrates representative TTC-stained coronal brain slices of controls and animals undergoing either reperfusion or craniectomy alone at 1 hour after MCA occlusion.

We did not observe a significant difference in total brain volume among all groups (data not shown; lowest brain volume was 1257 ± 94 mm³ in group 3; highest was 1334 ± 80 mm³ in group 11).

Infarct volume in animals undergoing reperfusion at 1 hour after MCA occlusion (79 ± 59 mm³) was significantly smaller than that in control animals (225 ± 26 mm³) (\( P < 0.01 \)). Reperfusion at 4 hours (182 ± 62 mm³) and 12 hours after MCA occlusion (231 ± 69 mm³) did not result in a significant reduction of infarct volume (\( P > 0.05 \)). Two of 12 animals...
Reperfusion at 4 hours after MCA occlusion resulted in significantly smaller infarct volume than that in control animals (102±36, 135±35, and 188±51 mm³, respectively) (P<0.05). None of these animals showed intraparenchymal hemorrhage. Reperfusion and craniectomy at 4 hours after MCA occlusion resulted in a reduction of infarct volume of 26% compared with reperfusion at 4 hours (135±35 versus 182±62 mm³) but yielded no significant additional benefit compared with craniectomy alone (109±39 mm³).

Discussion

In addition to reperfusion, craniectomy, an old neurosurgical concept for the treatment of increased ICP, has been shown to be effective in a few experimental studies7–9 and in selected patients with large MCA infarction.6,10,11 Theoretically, a combination of reperfusion and craniectomy may be beneficial because a major mechanism of reperfusion injury, ie, exacerbation of brain edema with elevation of ICP,16,21–23,32–34 could potentially be counterbalanced by craniectomy. Moreover, because the exact mechanism by which craniectomy can rescue ischemic brain tissue is yet unclear, it may be of interest to compare the efficacy of reperfusion and craniectomy in acute ischemic stroke.

We designed the present study to investigate the singular and combined effects of craniectomy and/or reperfusion at different time points after MCA occlusion on infarct volume and neurological outcome.

A comparison of TTC-derived infarct volume, loss in body weight, and neurological score at 7 days after MCA occlusion yielded the following results: (1) Early reperfusion at 1 hour after MCA occlusion significantly reduced infarction volume and improved neurological outcome compared with control. Reperfusion at later time points had no significant benefit compared with control. Two of 12 animals (16.7%) undergoing late reperfusion at 4 hours and 5 of 10 animals (50%) undergoing reperfusion at 12 hours revealed intraparenchymal hemorrhagic transformation; 3 of them had infarct volumes >300 mm³. These findings were interpreted as reperfusion injury. (2) All animals treated by craniectomy at 1, 4, or 12 hours survived, and infarct volume and neurological outcome were significantly better than those of controls, indicating that the therapeutic time window for beneficial craniectomy is longer than that for reperfusion. Neurological outcome was improved in a time-dependent fashion, with animals treated by craniectomy at 1 hour demonstrating the best results. (3) The combination of early reperfusion and craniectomy at 1 hour yields no additional benefit compared with reperfusion or craniectomy alone. Combined treatment at 4 hours achieved a reduction in infarct volume of 26% compared with reperfusion alone. When compared with craniectomy alone at 4 hours, combined treatment yields no significant additional benefit in terms of infarct volume and neurological outcome.
Results 1 and 2 are well known. When blood flow is restored after brief episodes of focal ischemia, infarct volume is smaller than after permanent vessel occlusion. In the rat suture model, the time window to reduce infarct volume is reported to be approximately 2 hours.\textsuperscript{14–19} Interestingly, although animals undergoing late reperfusion at 4 hours did not show significantly smaller infarct volume, their neurological score at day 7 was significantly better than that of control animals (2.8 versus 3.8). The high mortality in the control group (33%) may be a possible explanation for this finding because these animals had a score of 5 points, and none of the animals undergoing reperfusion at 4 hours died (the worst score was 4). In a recently published study, diffusion- and perfusion-weighted MRI was used to monitor the effects of craniectomy at 1 hour after MCA occlusion on infarct volume in rats.\textsuperscript{8} At 6 hours after MCA occlusion, diffusion-weighted MRI–derived hemispheric lesion volume in the craniectomy group was 23.0% compared with 44.1% in the untreated control group; additionally, craniectomy achieved significantly higher cortical blood flow than that in control animals. In a direct comparison with reperfusion at 1 hour, craniectomy was probably improved via activated MCA branches and leptomeningeal collaterals after decreasing ICP by decompressing the swollen brain; this improved cortical perfusion may be directly responsible, at least in part, for the reduced infarct volume.

In contrast to reperfusion, craniectomy does not have the risk of reperfusion injury. Yang and Betz\textsuperscript{23} demonstrated that 3 hours of focal ischemia followed by 3 hours of reperfusion in the rat produced more damage than 6 hours of permanent ischemia. Similarly, investigating the effects of temporary MCA occlusion in spontaneously hypertensive rats on neocortical infarct volume at 24 hours, Kaplan and colleagues\textsuperscript{15} found that focal ischemia for 3 hours followed by reperfusion resulted in larger infarct volumes compared with permanent MCA occlusion.

To investigate the combined effects of reperfusion and craniectomy, we chose reperfusion at 1 and 4 hours in combination with craniectomy at 1, 4, and 12 hours after MCA occlusion. Early reperfusion at 1 hour (a time point with maximal reperfusion effect) was chosen to test whether a combined treatment can achieve even better results. Additionally, we chose reperfusion at 4 hours (a time point at which no significant benefit was expected) to test whether a combination with craniectomy has any benefit.

In a previous study, Engelhorn and colleagues\textsuperscript{8} reported that a combination of reperfusion and craniectomy at 1 hour after MCA occlusion did not result in additional benefit compared with single treatment. However, the diffusion-weighted imaging–derived infarct volume was assessed only within the first 6 hours, and animals received 5 injections of contrast agent for cerebral perfusion measurement. Additionally, the TTC-derived infarct volume was assessed 24 hours after MCA occlusion. It is debatable whether these results may allow definite conclusions because this relatively short survival period may not assess final infarction volume.\textsuperscript{35} In our study 7 days after MCA occlusion, combined treatment at 1 hour did not result in significant smaller infarct volume or better neurological outcome compared with reperfusion or craniectomy alone. A possible explanation for this finding may be the relatively small edema volume after a 1-hour period of MCA occlusion associated with only a slight increase in ICP. Kawamura et al\textsuperscript{16} reported an edema volume of only 12 mm\textsuperscript{3} found in the basal ganglia and in the periphery of the pallidum after reperfusion at 1 hour compared with 47 mm\textsuperscript{3} found in control animals. Craniectomy may not be effective in these infarcts of the basal ganglia because craniectomy only significantly improves cortical perfusion.\textsuperscript{8}

All animals undergoing early reperfusion suffered infarction of the basal ganglia; infarcts of the MCA-supplied cortex, such as those observed in all control animals, were not found. In contrast, craniectomized animals suffered infarction of the basal ganglia, and, in a time-dependent fashion, the temporobasal MCA-supplied cortex was also infarcted (Figure 3). None of the craniectomized animals suffered infarction of the parietal cortex underlying the craniectomy site. This observation suggests that cortical perfusion after craniectomy was probably improved via activated MCA branches and leptomeningeal collaterals after decreasing ICP by decompressing the swollen brain; this improved cortical perfusion may be directly responsible, at least in part, for the reduced infarct volume.

Although the combination of reperfusion and craniectomy at 4 hours resulted in reduced infarct volume compared with reperfusion alone, this therapy yielded no significant additional benefit when compared with craniectomy alone at 4 hours.

When the effects of combined treatment are interpreted, some limitations must be considered. Animals treated by late craniectomy (groups 7, 10, 13) or reperfusion (group 4) 12 hours after MCA occlusion underwent a second administration of anesthesia and animal preparation. Although body temperature was maintained at 37°C to avoid hypothermia, it is unclear whether there was an additional beneficial or adverse effect of anesthesia.

All animals were killed 7 days after MCA occlusion. While this interval is long enough to correctly measure infarct volume, it is questionable whether neurological performance at this time point correlates with the final neurological outcome. In addition, we used a simple 5-point score (distinguishing only between no deficits; mild, moderate, and severe motor dysfunction; and death) to measure tendency of neurological performance. Still, there was good correlation between infarct volume and neurological performance ($r=0.68$).

Additionally, cerebral perfusion was not measured. It is unclear whether there was complete reperfusion in all animals after the suture was withdrawn or if there might have been no-reflow in some animals. Further studies using perfusion-weighted MRI or other techniques to evaluate perfusion alterations, such as autoradiography or radiolabeled microspheres, would be helpful to understand the underlying pathomechanisms and to transform the experimental results in the clinical setting.

In conclusion, our experimental results demonstrated that early reperfusion and craniectomy are both effective treatments in acute hemispheric stroke. While reperfusion at 4 and 12 hours was not beneficial and involved the risk of reperfusion injury, late craniectomy still resulted in significant...
benefit, indicating that the therapeutic time window for craniectomy is longer than that for reperfusion. Combined treatment of reperfusion and craniectomy yields no significant additional benefit compared with craniectomy alone.

References


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