Delayed Decompressive Surgery Increases Apparent Diffusion Coefficient and Improves Peri-Infarct Perfusion in Rats With Space-Occupying Cerebral Infarction

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Background and Purpose—There is no conclusive experimental support that decompressive surgery in late stages of space-occupying cerebral infarction will improve outcome. We studied the effects of delayed decompressive surgery on the development of tissue damage, edema formation, and cerebral perfusion with different MRI techniques in a rat model of space-occupying cerebral infarction.

Methods—Permanent middle cerebral artery (MCA) occlusion was performed in 6 Fisher 344 rats. Decompressive surgery was performed 17 hours after the occlusion. Each animal was assessed before surgery and 2 and 4 hours after surgery by means of diffusion-weighted T2-weighted, and flow-sensitive alternating inversion recovery perfusion-weighted MRI. Ischemic damage was also evaluated in hematoxylin-eosin-stained brain sections.

Results—Lesion volume as derived from apparent diffusion coefficient (ADC) maps decreased from 522 ± 98 mm³ before to 405 ± 100 mm³ (P = 0.016) 4 hours after decompressive surgery, whereas lesion volume from T2 maps increased from 420 ± 66 mm³ before to 510 ± 92 mm³ (P = 0.048) 4 hours after decompressive surgery. Midline shift decreased from 1.4 ± 0.1 mm to 0.5 ± 0.2 mm (P = 0.001). Blood flow in the noninfarcted area of the ipsilateral hemisphere improved from 25 ± 9 mL/min/100 g of tissue to 38 ± 9 mL/min/100 g of tissue (P = 0.035). Despite the pseudonormalization of ADC, irreversible damage was found in the entire MCA territory on histological evaluation.

Conclusions—In rats with space-occupying cerebral infarction, delayed decompressive surgery leads to a decrease in lesion volume derived from ADC maps, which is probably because of an increase of extracellular water formation. There are no signs that this reflects rescue of ischemic tissue. (Stroke. 2004;35:1476-1481.)

Key Words: animal models ■ brain edema ■ cerebral infarction ■ magnetic resonance imaging
toneal injection of 0.16 mg/kg fentanyl citrate, 5 mg/kg fluanisone, and 2.5 mg/kg midazolam, followed by subcutaneous injection of 0.05 mg/kg atropine sulfate. They were intubated and mechanically ventilated (Amsterdam infant ventilator MK3; Hoekloos) with 35% oxygen and 65% nitrous oxide. During all experiments, rectal temperature was maintained between 36.5°C and 37.5°C by means of a water heating pad. The left femoral artery was cannulated to monitor arterial blood pressure using a Datascop 3000 monitor (Datascop) and to obtain blood for arterial blood gas analysis before and after MCA occlusion (ABL 505/OSM3; Radiometer). When necessary, respiratory adjustments were made. After surgery, the animals received 0.3 mg/kg buprenorfin subcutaneously for relief of pain.

MCA occlusion was achieved by a minor modification of the intraluminal filament technique.11 In short, the right common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) were exposed through a ventral cervical midline incision. The pterygopalatine artery, ECA, and CCA were ligated with a 7.0 silk suture. A 3.0 prolene suture with a rounded tip and an intraluminal thread. Thereafter, the incision was closed.

Sixteen hours after MCA occlusion, the rats were neurologically examined according to the scale introduced by Bederson et al13 and refined by Menzies et al.14 Thereafter, they were intubated and ventilated with halothane 1% in a mixture of 35% oxygen and 65% nitrous oxide for MRI experiments and hemiacraniectomy. They remained under anesthesia during the surgical procedure and all measurements.

Decompresive surgery was performed 17 hours after MCA occlusion, as described by Forsting et al.8 Rats were immobilized in a stereotactic holder, the skull was exposed through a dorsal midline incision, and the temporal muscle was removed partially. A bone flap of 10 × 5 mm² was created in the parietal and temporal bone using a high-speed minidrill. The dura was opened by a large incision. Thereafter, the temporal muscle and the skin were adapted and sutured in place.

MRI Experiments

MRI experiments were performed just before, and at 2 and 4 hours after decompressive surgery on a 4.7 T Varian (Palo Alto, USA) horizontal bore spectrometer. Replicative form excitation and signal detection were accomplished by a Helmholz volume coil (diameter 9 cm, length 10 cm) and an inductively coupled surface coil (diameter 2 cm, respectively). A spin-echo sequence was used to determine the position of the animal (echo time [TE]/repetition time [TR]=40 ms/1 s; matrix [M]=[128×64]; field of view [FOV]=5.0×3.0 cm²; 21 1-mm-thick sagittal slices, number of excitations [NEX]=1). Eight contiguous 1.7-mm-thick transversal slices were planned, with the first slice 4 mm anterior to the center of the eyes. A single-scan diffusion-trace MRI sequence (4 b values: 100 to 1780 s/mm²; TE/TR=100 ms/2 s; M=128×64; FOV=3.2×3.2 cm²; NEX=2) was used to generate quantified images of the tissue water trace apparent diffusion coefficient (ADC). For DW imaging (DWI), a double spin-echo pulse sequence was used with 4 pairs of bipolar gradients with specific predetermined signs in each of the 3 orthogonal directions.15 The combination of gradient directions leads to a cancellation of all off-diagonal tensor elements, measuring the trace of the diffusion tensor. This provides unambiguous and rotationally invariant ADC values in 1 experiment. To minimize the high sensitivity of DWI to motion, the acquisition was triggered to the respiratory cycle.

T2W images were acquired using a multiecho sequence (TE/TR=17 ms+7×17 ms/3 s; M=256×128; FOV=3.2×3.2 cm²; NEX=2). To minimize interference at the slice boundaries, slices were acquired in alternating order.

PW imaging (PW1) was performed in a single slice through the infarct core (corresponding to slice number 5 from diffusion and T2W images) with use of flow-sensitive alternating inversion recovery (FAIR).16 A slice-selective inversion recovery image (Mns) and a nonselective inversion recovery image (M0) were acquired with turbo-fast low angle shot (FLASH) acquisition and a sufficient inversion time (TI) to allow inversion of labeled spins into brain tissue.17 MRI parameters were as follows: flip angle (α)=20°; TE/TR/TI=3 ms/6 ms/2000 ms; predelay=2.0 s (total TR=4.75 s); M=128×128; FOV=3.2×3.2 cm²; slice thickness=1.7 mm; NEX=96. For normalization of the FAIR signal, an axial magnetization (M0) image was acquired with the same parameters without inversion. For quantification of the FAIR signal, a slice-selective T1 map was acquired by Turbo-FLASH Look-Locker acquisition18 (α/TE/TR/TE=5/3 ms/11 ms; 10 TIs [0.4+9×1.4 s]; M=128×128; FOV=3.2×3.2 cm²; slice thickness=1.7 mm; NEX=8).

Data Processing and Analysis

All images were zero-filled to 256×256. ADC and T1 maps were generated by monoexponential fitting with interactive data language (IDL; Research Systems). Parametric images were analyzed in anatomic regions of interest using in-house software. Calculations of lesion volume were based on ipsilateral ADC or T2 differences of more than 20% compared with the mean value in the contralateral hemisphere. This threshold corresponds to a difference of more than 2 SDs but is less influenced by noise than the SD. Moreover, this threshold is close to the 23% drop in ADC found to correlate with ATP depletion 1 hour after MCA occlusion.19 Measurements of midline shift were performed with the software package ImageBrowser (SISCO/Varian) at the level of the infarct core, corresponding to slice number 5 of T2W images, according to the formula midline shift=total diameter−2×contralateral diameter, using the third ventricle as a landmark.

PWI data were processed with IDL. Relative FAIR images were obtained by subtracting the Mns from the M0 and dividing the result by the M0 image ([Mns−M0]/M0). T1 maps were obtained by monoexponential fitting with a correction for magnetization saturation.20 From these relative FAIR images and T1 maps, CBF maps in milliliters per minute per 100 g of tissue were calculated by T1 correction according to Calamante et al,21 assuming perfect inversion, a homogeneous blood–brain partition coefficient of 0.9 mL/g, and a blood T1 of 2 s. Further analysis was performed on region of interest (ROI) basis with ImageBrowser. Infarct ROIs were drawn in the T2 maps and defined as those regions with a 20% increase of T2 compared with the mean value of the contralateral hemisphere. Peri-infarct ROIs were defined as those covering the noninfarcted parts of the affected hemisphere, excluding the ventricles. Contralateral ROIs consisted of the whole contralateral hemisphere. These ROIs were transferred to the CBF maps, and CBF was determined in the infarct core, in the peri-infarct region, and in the contralateral hemisphere.

Statistical analyses of MRI parameters before and 2 and 4 hours after surgical treatment were performed by means of repeated-measures ANOVA. Outcome measures are expressed as means ± SD. Differences were considered significant at levels of P<0.05.

Histology

After the MRI experiments, the rats were killed by an intraperitoneal injection of 150 mg pentobarbital. The brains were removed and stored in a 4% phosphate-buffered formaldehyde solution. After dehydration in a phosphate-buffered 25% sucrose solution, coronal cryosections (25 μm) were cut and stained with hematoxylin and eosin for histopathological evaluation. Infarct was defined as the area of pallor caused by loss of affinity for hematoxylin affecting all cell types except infiltrated inflammatory cells. Cells were considered to be damaged irreversibly when the cytoplasm had become intensively eosinophilic, whereas the nucleus had become pyknotic, or when the cellular nucleic acids had completely lost their affinity for hematoxylin and eosin.
Results

Intraoperative physiological variables remained within the normal range (data not shown). Before hemicraniectomy, all rats had a neurological score of 4 according to Bederson et al, indicating spontaneous contralateral circling.\textsuperscript{13}

DW and T2W MRI

Before surgery, ischemic areas were visualized on ADC and T2 maps and covered the complete MCA territory in all animals (Figure 1). Mean ADC and T2 values of the contralateral hemisphere, threshold values, and values within the infarct and in peri-infarct tissue are presented in Table 1. Values of the contralateral hemisphere (and thereby threshold values) did not change significantly over time. Mean lesion volume as calculated from the ADC maps decreased from 522±98 mm$^3$ before to 458±92 mm$^3$ at 2 hours, and 405±100 mm$^3$ at 4 hours after surgery ($P=0.016$; Figure 2a). Of total lesion volume calculated from ADC maps, the percentage with increased ADC values increased from 4.6±2.7% to 10.4±6.7% ($P=0.05$). In contrast to ADC lesion volumes, lesion volume as deduced from T2 maps increased from 420±66 mm$^3$ before to 457±94 mm$^3$ at 2 hours, and 510±92 mm$^3$ at 4 hours after surgery ($P=0.048$; Figure 2b).

Midline Shift

Mean midline shift decreased from 1.4±0.1 mm before to 0.7±0.3 mm at 2 hours and 0.5±0.2 mm at 4 hours after surgery ($P=0.001$; Figure 3). The total volume of the affected hemisphere as deduced from T2 maps increased from 767±19 mm$^3$ to 820±58 mm$^3$ at 2 hours after surgery and 830±52 mm$^3$ at 4 hours after surgery ($P=0.033$).

PWI

Of 18 FAIR experiments, the results of 5 could not be analyzed because of poor signal-to-noise ratios as a result of low global CBF. The calculated perfusion maps of the remaining experiments showed regions with perfusion deficits covering the entire MCA territory. Figure 1C shows an example of a perfusion map before and 4 hours after surgery. For 5 of 6 rats, perfusion before and after surgery could be analyzed. The mean CBF in the peri-infarct region increased from 25±9 mL/min/100 g of tissue before to 37±9 mL/min/100 g of tissue 2 hours, and 38±5 mL/min/100 g of tissue 4 hours after surgery ($P=0.035$; Figure 4). There were no differences in CBF of the infarcted region and the contralateral hemisphere before and after decompressive surgery (14

\begin{table}[h]
\centering
\caption{ADC and T2 Values of the Contralateral Hemisphere, Threshold Values, and Values Within the Lesion and in Peri-Infarct Tissue}
\begin{tabular}{lllll}
\hline
 & Before Surgery & 2 Hours After Surgery & 4 Hours After Surgery & \(P\) \\
\hline
ADC contralateral (×10\textsuperscript{−4} mm\textsuperscript{2}/s) & 7.48±0.5 & 7.31±0.4 & 7.49±0.5 & 0.4 \\
ADC threshold for decreased values (×10\textsuperscript{−4} mm\textsuperscript{2}/s) & 6.0±0.4 & 5.8±0.3 & 6.0±0.4 & 0.4 \\
ADC within part of lesion with decreased values (×10\textsuperscript{−4} mm\textsuperscript{2}/s) & 4.4±0.2 & 4.5±0.3 & 4.4±0.7 & 0.3 \\
ADC threshold for increased values (×10\textsuperscript{−4} mm\textsuperscript{2}/s) & 9.0±0.5 & 8.8±0.5 & 9.0±0.6 & 0.4 \\
ADC within part of lesion with increased values (×10\textsuperscript{−4} mm\textsuperscript{2}/s) & 10.6±0.5 & 11.1±0.4 & 12.1±1.6 & 0.8 \\
ADC peri-infarct (×10\textsuperscript{−4} mm\textsuperscript{2}/s) & 7.1±0.4 & 7.4±0.4 & 7.6±0.4 & 0.7 \\
T2 contralateral (ms) & 53±2 & 53±2 & 53±2 & 0.2 \\
T2 threshold value (ms) & 63±3 & 64±5 & 64±3 & 0.6 \\
T2 within lesion (ms) & 80±4 & 81±3 & 83±3 & 0.5 \\
T2 peri-infarct (ms) & 51±1 & 52±2 & 52±2 & 0.6 \\
\hline
\end{tabular}
\end{table}

Data are expressed as mean±SD.
mL/min/100g tissue before and 12 mL/min/100 g of tissue 4 hours after surgery \( P = 0.6 \); 46 mL/min/100 g of tissue before and 44 mL/min/100 g of tissue 4 hours after surgery \( P = 0.4 \), respectively.

**Histology**

Signs of irreversible tissue damage, including cytoplasmic eosinophilia affecting both neurons and glial cells, clumping of nuclear chromatin, nuclear pyknosis, and ghost neurons, were observed in the entire MCA territory, including the parts where ADC had normalized. Cell density in the infarct compared with contralateral decreased with 40±14% in the cortex \( P = 0.001 \) and 43±14% in the striatum \( P = 0.001 \).

**Discussion**

In this model of space-occupying cerebral infarction, hemicraniectomy and durotomy performed when herniation was impending gave rise to a reversal of midline shift and an increase in blood flow in the noninfarcted tissue of the ipsilateral hemisphere. Apparent lesion volume as measured on ADC maps decreased, suggesting a beneficial effect on ischemic damage. However, there was an increase in lesion volume calculated from T2 maps, and histological evaluation demonstrated irreversible damage in the complete MCA territory, including areas with pseudonormalized ADC values.

ADC reductions in ischemic tissue are thought to be caused by cytotoxic edema formation and the associated decrease in extracellular water \(^2^3\) and have proved to be a very sensitive indicator of early ischemic brain damage. \(^2^4\) Several investigators have observed reversal of DWI abnormalities after transient focal ischemia followed by delayed recurrence. \(^2^4,^2^5\) It has been suggested that this phenomenon is caused by
secondary ischemic damage, after an initial recovery of the energy status. 25 This is the first report that describes an increase in ADC after hemicraniectomy in space-occupying cerebral infarction. Because irreversible injury was observed on histological evaluation in the entire MCA territory, including the parts where ADC had pseudonormalized, the decrease in apparent lesion volume on ADC maps did not indicate tissue recovery. Given the increase in apparent lesion volume as deduced from the T2 maps, and the increase in volume of the entire hemisphere, we suggest that the increase of ADC values may be caused by an increase of extracellular space.

T2 prolongation of brain tissue water, measured by T2W MRI, is thought to reflect vasogenic edema and is probably most pronounced in irreversibly damaged tissue. 26 An increase in extracellular space in our model may be explained by a decreased interstitial pressure caused by the bony decompression. This is in line with earlier findings. Cooper et al showed that bony decompression resulted in exacerbation of edema in a cold lesion model in dogs.27 In a rat model of MCA infarction, Engelhorn and colleagues found that lesion volume after a combination of reperfusion and decompressive surgery was significantly larger than lesion volume after reperfusion only.9

Analysis of the CBF maps revealed a region with a clear perfusion deficit covering the entire MCA territory in each animal. After surgery, the area with the perfusion deficit slightly increased, probably as a result of the increase of lesion volume by edema. CBF in the noninfarcted parts of the ipsilateral hemisphere improved significantly, supporting 1 of the rationales of decompressive surgery, which is to revert brain tissue shifts and to decrease ICP to improve cerebral perfusion.5,6 However, in contrast to a previous study,9 we observed no increase in CBV in the territory of the occluded artery. In this previous study, treatment was applied 1 hour after MCA occlusion, when there was no evidence of edema formation yet. Therefore, it is unlikely that delayed hemicraniectomy as performed in our study (and in most patients), exerts a beneficial effect by saving penumbral tissue in the area of the occluded artery. A beneficial effect of delayed decompressive surgery, if present, may be explained by the increase of CBF in the nonaffected vascular territories, thereby preventing recruitment of these territories in the infarct.

We did not compare ADC, T2, and CBV values after decompressive surgery with those in controls. However, in a previous study on the natural course of tissue damage in rats with space-occupying infarction, we did not observe normalization of ADC values nor reversal of midline shift and increase of CBV in the peri-infarct region in the time period between 16 and 24 hours after MCA occlusion.

In conclusion, delayed decompressive surgery might be beneficial by reversal of tissue shifts, with improvement of CBF in vascular territories surrounding the infarct. We found no beneficial effect of surgery on infarcted tissue within the MCA territory. Therefore, the intervention may only prevent additional damage in already severely affected patients. Randomized clinical studies focusing on functional outcome are required before implementing this strategy as a standard treatment modality.28,29

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References


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