Noninvasive Quantification of Brain Edema and the Space-Occupying Effect in Rat Stroke Models Using Magnetic Resonance Imaging

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Background and Purpose—Brain edema is a life-threatening consequence of stroke and leads to an extension of the affected tissue. The space-occupying effect due to brain edema can be quantified in rat stroke models with the use of MRI. The present study was performed to test 2 hypotheses: (1) Can quantification of the space-occupying effect due to brain edema serve as a noninvasive measure for brain water content? (2) Does morphometric assessment of brain swelling allow determination of true infarct size on MRI after correction for the space-occupying effect of edema?

Methods—Thirty rats were subjected to permanent suture middle cerebral artery occlusion. MRI was performed after 6 or 24 hours, and hemispheric swelling was assessed morphometrically. Interobserver and intraobserver agreements were determined for MRI measurements. In study I, the space-occupying effect due to brain edema was correlated with the absolute brain water content by the wet/dry method. In study II, lesion volumes corrected and uncorrected for edema were calculated on MRI and on TTC staining and compared.

Results—Interobserver and intraobserver agreements for MRI measurements were excellent ($r=0.97$). Brain water content and hemispheric swelling correlated well after 6 and 24 hours ($r=0.95$). Corrected lesion volumes correlated with $r=0.78$ between TTC staining and MRI. Without edema correction, lesion volumes were overestimated by 20.3% after 6 hours and by 29.6% after 24 hours of ischemia.

Conclusions—Morphometric assessment of hemispheric swelling on MRI can determine the increase in absolute brain water content noninvasively and can also provide ischemic lesion volumes corrected for brain edema. (Stroke. 2004;35: 566-571.)

Key Words: brain edema ■ cerebral infarction ■ magnetic resonance imaging ■ stroke, experimental

Brain edema is a common and life-threatening consequence of brain infarction. Focal ischemia causes a breakdown of the blood-brain barrier that leads to a net influx of water into the affected tissue (vasogenic brain edema) and causes an extension of ischemic brain lesions. This space-occupying effect causes displacement of midline structures and can lead to cerebral herniation and death. Mechanical compression of adjacent brain structures further reduces local cerebral blood flow and thus can cause additional ischemic damage.

In the present study we evaluated a noninvasive morphometric technique that allows quantification of the space-occupying effect of brain edema in a rat stroke model, using T2-weighted MRI. This method has 2 useful applications in experimental stroke research. The first application is quantification of brain edema. Assessment of hemispheric volumes on MRI allows a direct quantification of the space-occupying effect in experimental stroke. If it is assumed that the space-occupying effect is predominately caused by brain edema, this method furthermore provides an indirect quantitative measure of the brain water content (BWC). Because of its noninvasive character and simple applicability, this technique allows study of the temporal propagation of the ischemic lesion and concomitant brain edema simultaneously. This may provide new insights into the dynamic pathophysiology of vasogenic brain edema in the acute phase of stroke.

The second application is calculation of edema-corrected lesion volume (LV). The exact determination of ischemic LV is crucial for many experimental stroke studies. Lesion size, however, needs to be corrected for the space-occupying effect of brain edema to avoid an overestimation of the affected tissue volume. Edema correction is routinely performed on postmortem analysis (eg, 2,3,5-triphenyltetrazolium chloride [TTC] staining or histopathology) but usually not on MRI studies.
With the use of the suture middle cerebral artery (MCA) occlusion (MCAO) technique, the reliability and validity of quantification of the space-occupying effect and brain edema on MR scans were assessed by comparison with 2 postmortem reference methods (absolute BWC, assessed by the wet/dry method, and hemispheric swelling on TTC staining). The impact of edema correction on the assessment of LV was analyzed by comparison of edema-corrected and uncorrected LV at 2 different time points (6 or 24 hours) after induction of ischemia. Furthermore, we determined the interobserver and intraobserver reproducibility of morphometric MRI measurements.

**Materials and Methods**

**Animal Preparation**

All procedures used in this study are in accordance with our institutional guidelines. Male Sprague-Dawley rats, weighing 290 to 350 g, were anesthetized with 5% isoflurane for 2 minutes. Anesthesia was maintained with 2% to 3% isoflurane delivered in air at 3 L/min during surgery. PE-50 polyethylene tubing was inserted into the common carotid artery. The right common, internal, and external carotid arteries were exposed, and the external carotid artery was ligated. A 4-0 silicone-coated nylon suture was introduced through the common carotid artery. The occluder was advanced into the internal carotid artery 17.0 to 18.5 mm beyond the carotid bifurcation until an elastic force to extend left forepaw fully, 2 =inconstant circling to the left, 3 =constant circling to the left, 4=falling to the left, 5=no spontaneous walking with depressed level of consciousness, and 6=death.

**Clinical Score**

Neurological evaluation was performed 4 hours after induction of ischemia and scored on a 7-point scale: 0=no neurological deficit, 1=failure to extend left forepaw fully, 2=inconstant circling to the left, 3=constant circling to the left, 4=falling to the left, 5=no spontaneous walking with depressed level of consciousness, and 6=death.

**Magnetic Resonance Imaging**

The animals were fixed in a body restrainer and placed in an MRI spectrometer. The animals were anesthetized during imaging to minimize discomfort. Respiratory rate was monitored, and isoflurane concentrations were varied between 2.0% and 3.0% to keep the respiratory rate between 35/min and 45/min. Temperature was maintained at 37°C.

The spectrometer (Bruker PharmaScan; 7.0 T, 16 cm) operates at 300.51 MHz for 1 H imaging and is equipped with a 300-mT/m self-shielding gradient system. The linear polarized volume resonator (diameter 60 mm) was tuned and matched manually. Localizer images were acquired with the use of a spin-echo sequence. Fast Rapid Acquisition with Relaxation Enhancement (RARE) sequences (thickness 1 mm, repetition time [TR]=2500 ms, echo time [TE]=41.8 ms) were used to verify strictly symmetrical positioning and were repeated after correction of slice angulation, if necessary. High-resolution multislice, proton- and T2-weighted, double-contrast, spin-echo imaging was used to map hemispheric volumes. Sixteen contiguous coronal slices with a thickness of 2 mm were acquired (field of view 37×37 mm, matrix 512×256, TR=3000 ms, ET1=27 ms, ET2=72 ms, acquisition time=25.5 minutes, 2 averages).

To map the apparent diffusion coefficient (ADC) of water, diffusion-weighted images were acquired with a spin-echo sequence. Eight contiguous coronal slices were acquired (thickness 2 mm, field of view 37×37 mm, matrix 256×128, TR=2000 ms, TE=22.4 ms, acquisition time=17 minutes, 1 average, single echo). Four sets of coronal images were recorded to quantify ADC, with equidistant diffusion gradient values of 10, 50, 90, and 130 mT/m. With a duration of 9 ms and a separation time of 20.64 ms, this resulted in 4 b values of 49.0, 380.8, 1039.8, and 2026.0 s/mm². ADC maps were calculated by a least mean square fit with the use of the implemented Image Processing Tool.

Computer-aided planimetric assessment of the lesion and hemispheric volumes was performed by 2 blinded investigators. Hemispheric volumes were determined on T2-weighted imaging with the use of image analysis software (Image J 1.25s, National Institutes of Health). After enlargement and optimal adjustment of brightness and contrast, the hemispheres were traced manually on each slice. The position of the midline was determined with the use of the following neuroanatomic landmarks: falk cerebri, corpus pineale, fissura longitudinalis, infundibulum, aqueductus cerebri, and third ventricle (Figure 1). Lesion volumes were determined from ADC maps by computer-aided manual tracing of the hypointense lesions. The areas were then summed and multiplied by the slice thickness. LV was calculated with and without edema correction and expressed as percentage of the hemispheric volume. This calculation is based on 3 assumptions: (1) Compression of the contralateral hemisphere is comparable to compression of the entire healthy brain tissue, whereas the lesion is not compressed. (2) The contralateral hemisphere is compressed to the same extent as the affected hemisphere.

**Figure 1.** MRI and TTC staining. Representative T2 images (T2-I) and ADC maps 6 hours (left) and 24 hours (right) after MCAO. Ischemic lesions correspond closely between MRI and TTC staining. The space-occupying effect due to brain edema leads to a strong horizontal displacement of midline structures on MRI. After the brains were removed from the skull (TTC staining), the space-occupying effect caused an extension of the outer edges of the affected hemispheres, while the midline shift almost completely returned to normal. DW-I indicates diffusion-weighted imaging.
is extended. Total brain volume does not change. (3) Volume extension occurs only within the lesion, not in the unaffected tissue. With these assumptions, a compression factor $F$ may be defined as follows:

$$F = \frac{(HV_c + HV_i)/2}{HV_c + HV_i + LV^v - HV_c - HV_i}$$

where $LV^v$ and $LV^u$ indicate corrected and uncorrected lesion volumes, respectively, and $HV_c$ and $HV_i$ indicate volumes of the contralateral and ipsilateral hemisphere, respectively.

Solving this equation for LV corrected for edema ($LV^v$) results in the following:

$$LV^v = HV_c + HV_i - (HV_c + HV_i - LV^v) \cdot \frac{HV_c + HV_i - LV^v}{2HV_c}.$$  

The uncompressed volume of the contralateral hemisphere was calculated as follows:

$$HV^u = \frac{HV_c + HV_i}{2}.$$  

Therefore, the hemispheric LVs, uncorrected ($%HLV^u$) and corrected for edema ($%HLV^v$), were calculated as follows:

$$%HLV^u = \frac{2LV^v}{HV_c + HV_i} \cdot 100$$

$$%HLV^v = \frac{2LV^v}{HV_c + HV_i} - \frac{HV_c - HV_i + LV^v}{HV_c} \cdot 100.$$  

The space-occupying effect due to brain edema, expressed as the volume increase of the affected hemisphere ($%HSE$), was calculated with the use of Equation 3:

$$%HSE = %HLV^v - %HLV^u.$$  

### Postmortem Analysis

To analyze absolute BWC (study I), the convexities of the brains were separated into the ipsilateral and contralateral hemispheres. After wet weight was measured, the tissue was dried to a constant weight at 50°C and weighed again. The BWC ($%H_2O$) was calculated with the use of Equation 4:

$$%H_2O = \frac{\text{Wet Weight} - \text{Dry Weight}}{\text{Wet Weight}} \cdot 100$$

The increase of BWC of the affected hemisphere ($%H_2O$) was expressed as the difference in absolute BWC between both hemispheres, as follows:

$$%H_2O = %H_2O_{\text{ipsilateral}} - %H_2O_{\text{contralateral}}.$$  

To quantify LV and hemispheric swelling on TTC staining (study II), the brains were sectioned coronally into 6 slices (2 mm) and incubated in a TTC solution. The slices were then fixed in formalin and scanned (ScanJet 3400C, Hewlett-Packard; resolution 600×600 dpi). With the use of image analysis software (ImageJ1.25s), the areas of the infarcted regions and of both hemispheres were calculated for each slice. The ipsilateral and contralateral hemispheric volumes ($HV_c$ and $HV_i$) were calculated by multiplying the area by the slice thickness. Volume assessment was performed by an investigator blinded to group assignment.

The hemispheric LV without edema correction ($%HLV^v$) was calculated by using $HV_c$ as a reference since the contralateral hemisphere is not extended or compressed after the brain is removed from the skull, as follows:

$$%HLV^v = \frac{LV}{HV_c} \cdot 100.$$  

Edema-corrected hemispheric LV ($%HLV^v$) was calculated with the use of Equation 7, as follows:

$$%HLV^v = \frac{LV - \frac{HV_i - (HV_c - LV^v)}{HV_c} \cdot 100}{HV_c}.$$  

The space-occupying effect, expressed as the volume increase of the affected hemisphere ($%HSE$), was calculated with the use of Equation 8, as follows:

$$%HSE = \frac{HV_c - HV_i}{HV_c} \cdot 100.$$  

### Experimental Protocol

#### Pilot Study

MRI was performed in 4 healthy rats to determine reliability of slice angulation procedure. Hemispheric volumes were measured on each slice, and side-to-side differences were calculated.

#### Study I

This study was performed to determine the relation between the extent of the space-occupying effect due to brain edema ($%HSE$) and the difference in the absolute BWC between the hemispheres ($%H_2O$), with the use of the wet/dry method.

MRI was performed 6 or 24 hours after MCAO. The animals were killed immediately after imaging by decapitation under deep anesthesia. The brains were removed, and measurement of the absolute BWC was performed (Table 1).

#### Study II

Study II was performed to determine the reliability of our MRI method to calculate edema-corrected LV noninvasively by comparison with TTC staining.

MRI was performed 6 or 24 hours after MCAO. Animals were killed immediately after imaging, and TTC staining was performed. LV calculation from TTC staining was performed in group II after 24 hours but not after 6 hours because TTC staining does not provide reliable results early after onset of ischemia (Table 1).

### Interobserver and Intraobserver Reproducibility

We determined interobserver and intraobserver reproducibility of $%HSE$ measurement using MRI data from study I.

To assess interobserver reproducibility, $HV_c$ and $HV_i$ were measured by observer 1, with the second set of measurements performed 2 months after the first set. To examine intraobserver reproducibility, observer 2 determined $HV_c$ and $HV_i$ on the same images, and side-to-side differences were calculated with the first and second set of measurements performed by observer 1. Observer 2 was blinded to the results of observer 1. Both observers were blinded to group assignment.

### Statistical Analysis

We determined interobserver and intraobserver reproducibility using the Pearson correlation. The Mann-Whitney U test was used to compare parametric and nonparametric data. A probability value $<0.05$ was considered significant.

### Results

#### Pilot Study

Validity of angulation correction was verified in healthy rats. Side-to-side differences of hemispheric volumes were negligible (mean, 0.01±0.46%; range, −0.96% to 0.83%) among the slices, indicating a precise positioning procedure.

#### Clinical Findings and Physiological Parameters

One animal died during 24-hour imaging, and 1 rat suffered a subarachnoid hemorrhage. These animals were excluded and replaced.

Physiological parameters (body weight, mean arterial blood pressure, PO$_2$, PCO$_2$, pH, and body temperature) did not
differ among the groups ($P>0.05$) and were within the physiological range (data not shown). All animals showed clinical signs of moderate to severe hemiparesis 4 hours after MCAO. The clinical score ranged from 1 to 4 (median 2) and did not differ significantly between the groups ($P>0.05$).

**Interobserver and Intraobserver Reproducibility**

Interobserver and intraobserver reproducibility, as determined for MRI measurement of $\%\text{HSE}$, $\%\text{HLV}^u$, and $\%\text{HLV}^e$ at 6 and 24 hours after MCAO, was excellent, with correlation coefficients ranging from 0.97 to 0.99 (Table 2).

**Correlation Between Space-Occupying Effect on MRI ($\%\text{HSE}$) and Absolute BWC (Study I)**

The absolute BWC of the ipsilateral hemisphere, determined by the wet/dry method, was 78.08±0.24% after 6 hours and 80.08±1.41% after 24 hours of ischemia, while the BWC of the contralateral hemispheres was similar in both groups (76.08±0.68% after 6 hours and 75.89±0.74% after 24 hours). Therefore, the increase of absolute BWC of the affected hemisphere ($\Delta\text{H}_2\text{O}$) was calculated to be 2.00±0.57% after 6 hours and 4.19±1.06% after 24 hours of MCAO.

The space-occupying effect ($\%\text{HSE}$) derived from MRI was highly correlated with $\Delta\text{H}_2\text{O}$ (6 hours: $r=0.92$; $P<0.005$; 24 hours: $r=0.95$; $P<0.001$ (Figure 2).

**Space-Occupying Effect ($\%\text{HSE}$): Comparison Between MRI and TTC**

To determine the validity of MRI measurements for calculation of the space-occupying effect, TTC staining was used as a reference method. After 24 hours of ischemia, $\%\text{HSE}$ was 18.3±2.4% on MRI and 16.3±2.4% on TTC staining, and there was a close correlation between the 2 techniques ($r=0.86$; $P<0.05$) (Figure 3). TTC staining quality was not sufficient after 6 hours; $\%\text{HSE}$ was therefore calculated from MRI only and was 9.2±2.6% at this time.

**Lesion Volume Calculation: Impact of Edema Correction**

Study II was performed to determine the impact of edema correction on the calculation of infarct size after 6 and 24 hours of ischemia. TTC staining was used as a reference method in the 24-hour group.

After 6 hours, $\%\text{HLV}$ was calculated to be 45.8±21.0% without edema correction and 36.5±21.9% with edema correction on MRI. Without edema correction, LV was therefore overestimated by 20.3% (9.3%$\text{HLV}$).

After 24 hours of ischemia, lesion size determined by MRI was calculated to be 61.9±7.2% without edema correction and 43.6±6.0% with edema correction. Hemispheric LV was therefore overestimated by 29.6% (18.3%$\text{HLV}$) without edema correction.

TTC staining revealed lesion sizes of 57.9±9.4% without edema correction and 41.6±7.7% with edema correction. Without edema correction, $\%\text{HLV}$ was likewise overestimated by 28.2% (16.3%$\text{HLV}$) (Figure 4).

Corrected lesion size correlated well between MRI and TTC staining ($r=0.78$; $P<0.05$).

**Discussion**

In the present study we evaluated a method, modified from Loubinoux et al., for quantification of the space-occupying effect of brain edema in acute experimental stroke noninvasively, using MRI. This method has 2 useful applications in experimental stroke research, as discussed below.

### TABLE 1. Study Protocol

<table>
<thead>
<tr>
<th>Study</th>
<th>Purpose</th>
<th>Methods</th>
<th>Animals Sacrificed</th>
<th>Sample Size</th>
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<td>Study I</td>
<td>To determine the relation between the space-occupying effect due to edema and the absolute brain water content.</td>
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<td>n=7</td>
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<td>Study II</td>
<td>To determine the reliability of morphometric edema-corrected LV calculation on MRI- vs TTC (after 24 h only)</td>
<td>MRI vs 6 h after MCAO</td>
<td>n=7</td>
<td>n=7</td>
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### TABLE 2. Interobserver and Intraobserver Reliability of MRI Measurements 6 and 24 Hours After MCAO

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<th>6 Hours of Ischemia</th>
<th>24 Hours of Ischemia</th>
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<tbody>
<tr>
<td></td>
<td>$%\text{HSE}$</td>
<td>$%\text{HLV}^u$</td>
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<tr>
<td>Intraobserver reproducibility</td>
<td>$r$=0.97</td>
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<td>$P$</td>
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</tr>
<tr>
<td>$P$</td>
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Calculation of Edema-Corrected Lesion Volume
Accuracy in measurement of infarct volume in experimental brain research is confounded by postischemic brain edema, which increases the volume of the affected tissue and leads to an overestimation of lesion size. Thus, edema-corrected LV calculation was an established method in postmortem analysis (eg, TTC staining or histopathology) but has been widely neglected in the field of noninvasive brain imaging, although a considerable amount of vasogenic edema is detectable as early as 2 hours after MCAO.

For the calculation of uncorrected and corrected LV, planimetric assessment of the lesion and the ipsilateral and contralateral hemispheres is required. Modern MRI scanners allow display of midline structures clearly on T2-weighted images and thus delineation of both hemispheres separately (Figure 1). Ischemic lesions can be depicted easily on DWI early after onset of ischemia. Resolution of DWI, however, is limited for the delineation of midline structures. Thus, we calculated hemispheric volumes from T2-weighted images and LV from diffusion-weighted images. Exact assessment of the hemispheric volumes presupposes precise right-angled slice positioning. Slice angulation was therefore carefully corrected.

Calculation of edema-corrected LV on MRI was performed with the use of an equation modified from Loubinoux et al., which considers (1) that edematous tissue compresses unaffected tissue of the ipsilateral and contralateral hemispheres to the same extent and (2) that an extension of the outer edges of the affected hemisphere does not occur, since the brain is located within the intact skull. Therefore, different equations were used for LV calculation on MRI and on TTC staining. Ex vivo, the midline shift almost returned to normal after the brains were removed from the skull, indicating absence of compression of the unaffected tissue by the edematous lesion.

The space-occupying effect here simply results in an extension of the outer edges of the ipsilateral hemisphere without affecting the contralateral side.

The excellent interobserver and intraobserver agreement for %HLV, %HLV, and %HSE indicates that hemispheric volume calculation can be performed in a highly reproducible fashion. Thus, edema-corrected LV can be calculated.

The present study indicates that brain edema contributes to approximately 30% of LV 24 hours after ischemia on MRI. TTC staining has been used as a reference method and likewise indicated an overestimation of LV of 28% if edema correction was not performed. This finding is in accordance with MRI studies from Loubinoux et al., which demonstrated a 25% increase in LV due to brain edema 24 hours after ischemia. Determination of hemispheric swelling on TTC staining can lead to a slight underestimation of the space-occupying effect because formalin fixation causes a shrinkage of the tissue that might be more pronounced within the lesion compared with healthy tissue, as a result of the difference in BWC. However, since the difference in BWC is small (2% to 4%), this effect may be negligible.

Vasogenic edema is detectable very early (<2 hours) within the ischemic tissue. Its contribution to LV as a result of the space-occupying effect, however, is surprisingly profound at early time points. At 6 hours after MCAO, the LV was already increased by 20% because of brain edema. This finding is in contradiction to previous statements that edema volume may be negligible at early time points with respect to LV calculation.

Estimation of Increase in Absolute BWC
The difference between corrected and uncorrected LV, furthermore, provides an indirect measure for the space-occupying effect (%HSE), which is predominately caused by vasogenic brain edema. We evaluated this technique by comparison with 2 reference methods: corrected and uncorrected LV were obtained from TTC staining, in which edema correction was widely accepted. After 24 hours, MRI and TTC staining showed an almost exact agreement of uncorrected and corrected LV. TTC staining, however, is not reliable early after stroke onset because of insufficient infarct demarcation at this point and therefore was not performed after 6 hours. To support the hypothesis that the space-occupying effect is predominately caused by brain edema, we measured the side-to-side difference in absolute BWC (%ΔH2O) in a second study, using the wet/dry method. The excellent correlation between %ΔH2O and %HSE at both time points (6 and 24 hours) proved %HSE as a reliable noninvasive measure for the increase in absolute BWC and
Thus for the amount of brain edema within the affected hemisphere.

Previous approaches to measurement of in vivo BWC with the use of T1- and T2*-weighted imaging provided linear relationships of MRI quantification and ex vivo wet/dry measurement with correlation coefficients ranging from \( r = 0.79 \) to \( r = 0.98 \).\(^{16,17}\) Estimation of the increase of BWC, based on our morphometric method, however, is much easier to apply and does not require sophisticated postprocessing of MRI data. Our technique is comparable to these methods in terms of accuracy, but, in contrast, it allows calculation of LV corrected for the space-occupying effect due to edema.

In conclusion, morphometric assessment of hemispheric swelling correlates well with absolute BWC and swelling on TTC staining. This method allows calculation of ischemic LV corrected for the space-occupying effect of brain edema on MR images. Without correction for edema, lesion size is overestimated by almost 30% after 24 hours and by 20% after 6 hours of MCAO. These findings emphasize that edema correction is important for the exact determination of lesion size on MRI in both the subacute and the acute stages of cerebral ischemia, which is relevant particularly for the interpretation of neuroprotective drug studies.

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**References**