Edema formation in the hyperacute phase of ischemic stroke

Laboratory investigation

TIJO GERRIETS, M.D.,1,3 MAUREEN WALBERER, D.V.M.,2,3 NOUHA RITSCHEL, D.V.M.,3 MARLENE TSCHERNATSC, M.D.,1 CLEMENS MUELLER, PH.D.,3,4 GEORG BACHMANN, M.D.,3,4 MARKUS SCHEUNGR, M.D.,3,5 MANFRED KAP, M.D.,1,3 AND MAX NEDELMANN, M.D.1,3

1Department of Neurology, Justus Liebig-University Giessen; 2Department of Neurology, University Hospital of Cologne; 1Experimental Neurology Research Group, Justus Liebig-University Giessen and Kerckhoff-Klinik; and Departments of 4Radiology and 5Cardiac Surgery, Kerckhoff-Klinik, Bad Nauheim, Germany

Object. Brain edema formation is a serious complication of ischemic stroke and can lead to mechanical compression of adjacent brain structures, cerebral herniation, and death. Furthermore, the space-occupying effect of edema impairs regional cerebral blood flow (rCBF), which is particularly important in the penumbra phase of stroke. In the present study, the authors evaluated the natural course of edema formation in the hyperacute phase of focal cerebral ischemia.

Methods. Middle cerebral artery occlusion (MCAO) or a sham procedure was performed in rats within an MR imaging unit (in-bore occlusion). Both pre- and postischemic images could be compared on a pixel-by-pixel basis. The T2 relaxation time (T2RT), a marker for brain water content, was measured in regions of interest.

Results. A significant increase in the T2RT was detectable as early as 20–45 minutes after MCAO. At this early time point the midline shift (MLS) amounted to 0.214 ± 0.092 cm in the MCAO group and 0.061 ± 0.063 cm in the sham group (p < 0.007). The T2RT and MLS increased linearly thereafter. Evans blue dye was intravenously injected in additional animals 20 and 155 minutes after MCAO. Extravasation of the dye was visible in all animals, indicating increased permeability of the blood-brain barrier.

Conclusions. Vasogenic brain edema occurs much earlier than expected following permanent MCAO and leads to MLS and mechanical compression of adjacent brain structures. Since compression effects can impair rCBF, early edema formation can significantly contribute to infarct formation and thus represents a promising target for neuroprotection. (DOI: 10.3171/2009.3.JNS081040)

Key Words • stroke • brain edema • magnetic resonance imaging • neuroprotection • rat

Occlusion of a cerebral artery typically leads to a centrifugal propagation of cell death within the hypoperfused brain tissue (the penumbra). Although the speed of infarct dispersion is difficult to determine and may differ considerably among individual patients, it is generally accepted that the majority of “tissue at risk” becomes irreversibly damaged within the first few hours after the onset of stroke.15,26

Abbreviations used in this paper: ACA = anterior cerebral artery; ADC = apparent diffusion coefficient of water; AQP = aquaporin; BBB = blood-brain barrier; CCA = common carotid artery; ICA = internal carotid artery; MLS = midline shift; rCBF = regional cerebral blood flow; ROI = region of interest; T2RT = T2 relaxation time.

The definite size of the ischemic lesion depends on the spatial extent and severity of hypoperfusion during this phase. We hypothesized that the perfusion deficit is determined by not only the lack of blood supply due to artery occlusion but also the formation of vasogenic brain edema, which leads to mechanical compression of the adjacent microvessels and veins.29,30 This hypothesis is supported by data from several animal studies indicating a significant improvement in rCBF and a marked reduction in the infarct size after decompressive hemiepithomie.4–6,14

The timing of vasogenic edema formation is important in this context. If mechanical compression effects on rCBF essentially contributed to infarct growth during the penumbra phase, significant brain swelling would be ex-
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expected to occur in the first hours of stroke. In this case, very early treatment of edema formation might provide neuroprotective effects by improving CBF in the vicinity of the lesion and thus reducing infarct size.

Because data about the natural time course of brain swelling in the hyperacute phase are lacking, we conducted an in vivo experiment to quantify edema formation and the resulting space-occupying effect within the first 3 hours of stroke.

Methods

All procedures were in accordance with the German animal protection legislation.

Anesthetic Technique

Twelve male Sprague-Dawley rats were anesthetized with 5% isoflurane delivered in air at 1.0 L/minute. During surgery, anesthesia was maintained with 2.5% isoflurane delivered in air at 0.5 L/minute. Throughout the surgical procedure, blood pressure was monitored using polyethylene tubing (PE-50) inserted into the left femoral artery, also making blood samples available for the measurement of blood gases. Body core temperature was maintained at 36.5–37.0°C by using a thermostatically controlled heating pad.

Surgical Procedure

After a midline incision in the ventral neck, the right CCA and the relevant cranial branches were exposed. The external carotid artery and the pterygopalatine branch of the ICA were ligated with a 5-0 suture. The polyethylene tubing was filled with heparinized saline and 6 TiO2 macrospheres (diameter 0.315–0.355 mm, BRACE). For the sham procedure, only heparinized saline was used. The tubing was then inserted into the CCA, with its tip placed distally to the origin of the pterygopalatine artery, and fixed with a 5-0 suture.

Magnetic Resonance Imaging

Subsequent to surgery, the rats were fixed in a body restrainer for MR imaging. The head was positioned in a cone-shaped head holder with an additional tooth bar. A pressure probe was placed underneath the thorax to enable monitoring of the respiratory rate during the entire procedure.

Keeping the respiratory rate between 50 and 60 breaths/minute, anesthesia was sustained with 1.5–2.5% isoflurane delivered in air at 0.5 L/minute. Body temperature was maintained at 36.5–37.0°C with a thermostatically controlled, water-filled, heating reel. Before positioning the animals into the MR imaging unit, the body core temperature, arterial blood pressure and pH, pCO2, and pO2 were determined.

For imaging, the restrainer was positioned into a custom-designed linear polarized volume resonator and tuned and matched manually. Localizer images were acquired using a spin echo sequence (TR 150 seconds, TE 12 msec, and slice thickness 4 mm) with 3 orthogonal slices. Each animal’s position could be optimized by shifting or tilting the resonator as necessary.

To map the ADCs, diffusion weighted MR images were acquired with the use of a fat-suppression echo planar sequence. A volume shim with a volume-selective double spin echo sequence (TR 1 second, TE 30 msec, and voxel size 10 × 10 × 15 mm3) was performed before the acquisition of the first diffusion series to optimize image quality. The achieved full-width at half-maximum of the water line was ~ 25–35 Hz. Six contiguous, coronal slices with a thickness of 2 mm were collected: field of view 32 × 32 mm, matrix 128 × 128, TR 3003 msec, TE 38.6 msec, number of excitations 4. Fourfold segmentation was used to reduce imaging artifacts due to local field inhomogeneity. Five sets of coronal images were recorded for ADC quantification, with equidistant diffusion gradient values of 10, 40, 70, 100, and 130 mT/m. A diffusion gradient duration (δ) of 9 msec and a gradient separation time (Δ) of 15 msec resulted in the 5 following b values (expressed in seconds/mm2): 6.96, 111.3, 340.8, 695.6, and 1175.5. The acquisition time for each echo planar imaging sequence was 4.5 minutes. The ADC maps were calculated by a least-squares fit using Image Processing Tool of the ParaVision software (version 2.1, Bruker).

A Carr-Purcell-Meiboom-Gill spin echo imaging sequence was used to map the T2RT. Sixteen slices (thickness 2 mm) were acquired: field of view 37 × 37 mm, matrix size 512 × 256, TR 3833.5 msec, 90° excitation pulse, 180° rephasing pulse, number of excitations 1, 12 echoes: TE 18–216 msec, acquisition time 16.5 minutes. The T2RT maps were calculated by a least-squares fit (Image Processing Tool, ParaVision).

Diffusion weighted and T2 MR images obtained before MCAO were used to generate baseline values.

In-Bore MCAO

Using 0.2 ml of heparinized saline, the macrospheres were washed into the ICA to occlude the MCA (6 animals). For the sham procedure, the same amount of heparinized saline was injected (6 animals). Immediately after the injection, diffusion weighted imaging was performed, repeating a run every 5 minutes 4 times in a row followed by T2 MR imaging. The whole sequence was repeated another 3 times.

After imaging, the tubing was removed and the CCA was ligated to avoid hemorrhage. Animals were placed under a thermostatically controlled heating lamp until they recovered from the anesthesia.

Clinical Evaluation

Five hours after MCAO, a clinical evaluation was performed using a 6-point scale: 0 = no neurological deficit; 1 = failure to extend the left forepaw fully; 2 = circling to the left; 3 = falling to the left; 4 = no spontaneous walking together with a depressed level of consciousness; 5 = death,10,11,30

Postmortem Analysis

The animals were deeply anesthetized and killed, and each brain was examined to detect hemorrhage. Using a
magnifying glass, the position of the macrospheres inside the basal cerebral arteries was determined. If at least 1 macrosphere directly blocked the MCA mainstem or if the intracranial ICA and ACA were occluded, preventing blood flow into the MCA, then the MCAO was considered successful.

Evaluation of MR Imaging Data

The quantification of ADC was performed in ROIs within the center of the ischemic area on all imaging slices displaying ischemic lesions and at corresponding positions in the contralateral hemisphere. The position of the ROIs was defined on ADC maps obtained 150 minutes after MCAO, where the infarct was clearly visible. The difference in ADC between the ischemic and unaffected hemispheres was then calculated. For rats subjected to the sham procedure, ROIs were placed on Slices 3 and 5 in a position where ischemic lesions typically appear after macrosphere injection. The ADC was similarly measured on the images collected at early time points, while the ROI positions remained unchanged.

To quantify the T2RT, the procedure was repeated using the same ROI positions as those used for the ADC quantification.

Midline shift quantification was performed on the T2 images, where the position of the third ventricle could be clearly determined in all animals. The distance between the outer border of the cortex and the center of the third ventricle was measured from the ipsilateral (A) and contralateral sides (B), as described previously.\(^8,9,29\) Midline shift measurements were obtained at the level of maximum lateral displacement of the ventricle. Midline shift was calculated using the following equation, MLS = \(A - B / 2\).

Evans Blue Extravasation

The integrity of the BBB was assessed according to the extravasation of Evans blue dye in 15 additional animals. A 10% Evans blue dye (2 ml/kg) was slowly administered intravenously 20 (6 animals) or 155 minutes (6 animals) after MCAO or following the sham procedure (3 animals). The animals were killed 20 minutes after dye injection and were perfused with saline to clear the vascular bed of Evans blue. The brains were then removed, cut in coronal slices 2 mm thick, and scanned on a flatbed scanner at 600 dpi. Blood-brain barrier breakdown was diagnosed if Evans blue extravasation was detectable. The timing of these experiments was selected analogically to the first and last T2 imaging time points.

Statistical Analysis

Physiological parameters and clinical scores were compared between animals subjected to macrosphere injection and those that underwent the sham procedure by using the Student t-test or Mann-Whitney U-test. The ADC values and T2RTs were compared between ipsilateral and contralateral hemispheres using a Student t-test. Experienced investigators blinded to group assignments collected all data. A p value < 0.05 was considered statistically significant.

Results

Magnetic Resonance Imaging Experiments

Physiological parameters (body temperature, arterial blood pressure, and blood gas analysis) did not differ significantly between the MCAO group and the sham-operated animals (p > 0.05, data not shown).

Five hours after MCAO, the animals were fully recovered from anesthesia and displayed clinical signs of incomplete hemiparesis on the left side, with clinical evaluation scores ranging from 1 to 2. Animals that had undergone the sham procedure showed no abnormal neurological behavior (Score 0).

Already at the first ADC-imaging measurement (between 0 and 5 minutes after MCAO), the ADC was significantly decreased in MCAO animals compared with sham-operated animals (p = 0.02). The ADC continued to decline for ~1 hour thereafter and then remained almost stable.

The T2RT differed significantly between the MCAO and sham animals as early as the time of the first imaging sequence (20–45 minutes after MCAO), with higher values in animals with infarcts (p = 0.0009). During subsequent measurements, T2RT continued to increase in a linear fashion.

A significant MLS was detectable in the MCAO animals as early as the first T2RT measurement (20–45 minutes after MCAO; Fig. 1). The shift continued to increase linearly thereafter, paralleling the time course of T2RT (Fig. 2).

In the sham group no abnormal relaxation times or ADC values were noted.

No evidence of hemorrhage was found as the brains were removed from the skulls. In the MCAO group, inspection of the basal cerebral arteries revealed that the macrospheres were entrapped in the MCA mainstem and/or the distal ICA and ACA, blocking blood flow to the MCA. No macrospheres were found in the distal branches of the MCA or ACA. No abnormalities appeared in the sham-operated rats.

Evans Blue Dye Extravasation

Slight Evans blue dye extravasation was detectable in all animals 20 and 155 minutes after permanent MCAO (Fig. 3). The coloring covered the ipsilateral MCA territory completely or partially. Other vascular territories were not affected. No dye extravasation was found in the sham-operated animals.

Discussion

Data in the present study indicated that significant MLS can be detected as early as 20–45 minutes after permanent MCAO. Brain swelling increased thereafter in a linear fashion. This surprising result contradicts current concepts regarding the timing of BBB breakdown and vasogenic edema formation, which many authors expect to occur not before 2–3 hours after the onset of focal ischemia. Furthermore, edema formation is widely regarded as the typical feature of ischemia/reperfusion damage.
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rather than a complication following permanent vessel occlusion.26

Midline shift as a marker for a net volume increase in ischemic tissue is a result of vasogenic edema formation and correlates with absolute brain water content. In the clinical setting, lateral displacement of midline structures correlates well with the level of consciousness25 and predicts the risk of cerebral herniation and death in severe MCA-territory stroke.8,9,22 Midline shift in patients can easily be assessed with computed tomography, MR imaging, or ultrasonography.8,9,22,27 In animal studies, however, in vitro morphometric techniques to detect brain swelling, such as MLS quantification, are difficult to perform because the brain must be removed from the skull for histopathological evaluation, which changes its natural geometry. In addition, preparation of brain slices for microscopy alters the tissue water content, which will bias the results. In the present study, we made use of a sophisticated MR imaging technique that allows in vivo imaging of the brain before and immediately after MCAO without changing the rat’s position inside the MR imaging unit (in-bore occlusion).23 This technique enables a pixel-by-pixel comparison of pre- and postischemia images and thus is extremely sensitive to even subtle changes in the brain’s geometry in the hyperacute phase of stroke. Moreover, vasogenic edema can be measured on MR imaging by quantifying the T2RT. This parameter reflects the number of protons within the sample and correlates well with the absolute tissue water content (as determined, for example, by wet/dry measurements).2,11,13,19 In the current experiment, T2RT increased significantly at the first T2 imaging period (20–45 minutes) and almost paralleled the time course of MLS (compare the upper and center graphs in Fig. 2). Therefore, our findings based on MLS quantification were reproduced through a second independent method.

Cytotoxic edema, in contrast to the vasogenic type, is defined as a shift of water from the extracellular to the intracellular compartment and thus is not expected to contribute to the space-occupying effect.10,11,13,19 In the present experiment, the ADC was used as a measure of cytotoxic edema. A significant decline in the ADC can be detected at the first ADC-imaging period (0–5 minutes after MCAO), which accords with data in the pertinent literature. In contrast to the time course for the MLS, the ADC declined in a hyperbolic fashion with the most dominant decay occurring within the first 60 minutes and an almost stable course during the remaining time (Fig. 2). Since cytotoxic edema does not lead to a net volume increase in the affected tissue, vasogenic edema must be the cause of brain swelling.

What Causes Brain Swelling in the Hyperacute Phase of Stroke?

It is well accepted that focal ischemia, particularly if it is followed by reperfusion, leads to severe damage of the BBB and allows water and macromolecules to pass from the vessels into the tissue.17,18,31 Breakdown of the barrier can therefore be assessed based on the extravasation of tracers, such as Evans blue dye, Gd, [14C]aminoisobutyric acid, sucrose, or others. However, many authors have reported that tracer extravasation is not detectable earlier than a few hours after vessel occlusion.1,3,20 In the present study, slight extravasation of the Evans blue dye was detectable as early as 20–45 minutes after permanent MCAO. This finding suggests an increased permeability of the BBB for macromolecules, since Evans blue is bound to albumin and corroborates our MR imaging results.

Gross breakdown of the BBB, however, might not be the only explanation for brain swelling in the hyperacute phase of stroke, particularly since the dye extravasation was only faint. Water and larger molecules can pass the BBB through selective channels or even actively, for ex-
Aquaporins, a recently discovered family of water channel proteins, seem to play a pivotal role in this context. Aquaporins-1, -4, and -9 have been identified as the main water channels in the brain. In particular, AQP-4 and -9, both located on astrocytes, are upregulated during ischemia and seem to be involved in edema formation and resorption under these pathological conditions. Ribeiro et al. have compared the time course of AQP-4 expression with brain swelling in a mouse model of transient (30 minutes) MCAO. These authors found biphasic brain swelling, with maximum swelling 1 hour after MCAO (30 minutes after reperfusion) and a second peak at 48 hours after MCAO. This time course was parallel with the expression of AQP-4 in the vicinity of the lesion, which was significantly increased as early as 1 hour after MCAO and similarly showed a second peak at 48 hours after MCAO. These data suggest that early edema formation may be associated with AQP-4 expression and that this mechanism may also be relevant for very early brain swelling.

Clinical Implications of Early Brain Swelling

Brain edema or the resultant space-occupying effect can be successfully treated with decompressive craniectomy, osmodiuretics such as mannitol, or drugs that specifically target BBB dysfunction. The
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initiation of antiedema therapy in the very early phase may reduce the space-occupying effect and thus improve rCBF during the penumbra phase of stroke. The importance of mechanical compression of the brain in this context has been recently demonstrated in a stroke experiment in rats: Animals were bilaterally craniectomized or underwent a sham operation before MCAO. Preventive decompression reduced infarct size by one-half and significantly improved clinical outcome. These findings suggest that 50% of the ischemic lesion may be attributed to mechanical compression caused by edema formation. This hypothesis is further supported by data from Karibe et al., who showed that treatment with mannitol reduces ischemic lesion volume and improves functional outcome in an animal model of ischemic stroke. Early treatment of brain edema can therefore be considered an effective neuroprotective measure. The potential of “secondary neuroprotection” by surgical or pharmacological edema treatment should be further explored in preclinical and clinical studies.

Conclusions

Data in the present study indicate that vasogenic brain edema occurs as early as 20–45 minutes after permanent MCAO and is accompanied by MLS at this early time point. The treatment of vasogenic edema in the hyperacute phase of stroke might therefore reduce mechanical compression of brain tissue and provide “secondary neuroprotection.”

Disclosures

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References


