A reproducible model of an epidural mass lesion in rodents. Part II: Characterization by in vivo magnetic resonance imaging

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Object. The goal of this study was to characterize a novel epidural space-occupying lesion caused by balloon expansion in rodents by using sequential in vivo magnetic resonance (MR) imaging.

Methods. Ten Sprague–Dawley rats were intraperitoneally sedated. A trephination was performed over the left parietal cortex to attach a balloon-expansion device, which was secured with dental cement. Measurements were performed using a 1.5-tesla MR imaging device to obtain sequential T2-weighted and diffusion-weighted (DW) sequences in the coronal plane. A three-dimensional, constructed interference in steady state sequence was used for calculation of the balloon volume. The animal’s temperature, heartbeat, and the arterial percentage of oxygen saturation were monitored continuously. After a baseline examination had been performed, the balloon was inflated for a 30-minute period until it reached a maximum volume of 0.3 ml; this procedure was followed by a period of sustained inflation lasting 30 minutes, balloon deflation, and a period of reperfusion lasting 3 hours. After perfusion fixation of the animals, morphometric analysis of the lesion size and examination of the percentage of viable neurons in the hippocampus were performed.

Magnetic resonance imaging allowed for the precise visualization of the extension and location of the epidural mass lesion, narrowing of the basal cisterns, and development of a midline shift. A white-matter focus of hyperintensity, consistent with brain edema, developed predominantly in the contralateral temporal lobe. During sustained inflation the volume of the balloon did not change and comprised 5 to 7% of total intracranial volume. During the same period the white-matter edema progressed further but no increased signal was revealed on DW images. After balloon deflation the brain reexpanded to the calvaria and imaging signs of raised intracranial pressure subsided. A cortical area of hyperintensity on T2-weighted images developed in the parietal lobe in the region of the former balloon compression. This area appeared bright on DW images, a finding that corresponded to an early cytotoxic edema. After deflation white-matter vasogenic edema in the temporal lobes regressed within 3 hours after reperfusion. The cortical edema in the parietal lobe and the ipsilateral basal ganglia became sharply demarcated. The histopathological results (that is, the extent of tissue damage) corresponded with findings of the authors’ companion investigation, which appears in this issue.

Conclusions. Magnetic resonance imaging allows for a precise and sequential in vivo monitoring of a space-occupying epidural mass lesion and visualizes the time course of vasogenic and cytotoxic brain edema. This rodent model of an epidural mass lesion proved to be reproducible.

Key Words • magnetic resonance imaging • mass lesion • trauma • ischemia–reperfusion • blood–brain barrier • balloon expansion • rat


Abbreviations used in this paper: CISS = constructed interference in steady state; DW = diffusion-weighted; MR = magnetic resonance; SaO2 = arterial percentage of oxygen saturation; SD = standard deviation; 3D = three-dimensional.
zin (0.06–0.08 mg/100 g body weight) and Ketanest (9–12 mg/100 g body weight), and additional doses over time if needed. Throughout the entire experiment, SaO₂, heartbeat, and rectal temperature were monitored continuously. Temperature was kept between 36.5 and 37.5°C by applying a heating pad. The preparation of the animals and implantation of the balloon is described in Part I of this investigation,6 which appears in this issue, except that the balloon device was manufactured with an MR imaging–compatible plastic material (polyoxymethylene).

Experimental Protocol

Following baseline measurements of brain structures, performed using MR imaging, the epidural balloon was inflated with a constant flow rate of 0.6 ml/hour over a 30-minute period by using an infusion pump. This was followed by a 30-minute period of sustained inflation. The balloon was then deflated and the animals were monitored for a 3-hour period by using sequential MR imaging. After that, the animals were killed and perfusion fixed with a solution of 3.7% paraformaldehyde. Histopathological slices (7 μm thick) were stained with hematoxylin and eosin, hematoxylin, and cresyl violet. The percentage of viable neurons in the hippocampus was determined and the histological extent of tissue damage assessed in the manner described by Morikawa and colleagues17 by using a camera microscope and SigmaScan software.

Protocol for MR Imaging

Magnetic resonance imaging–based measurements were obtained using a 1.5-tesla MR imaging device with a round surface coil (diameter 10 cm). The animals were placed prone, lying on a heating pad. The head was centered in the coil and immobilized in a custom-made frame to avoid motion artifacts. Continuous measurements of T₂-weighted sequences obtained in the coronal plane with a slice thickness of 2 mm (TE 3500 msec, TR 96 msec, field of view 60 mm) were applied throughout the entire experiment. The acquisition time was 80 seconds. After every third T₂-weighted sequence, a DW sequence (free induction with steady precession sequence reversed in time, coronal plane, slice thickness 4 mm, field of view 200 mm, acquisition time 80 seconds) was applied in three orthogonal axes. At the end of the sustained inflation, a 3D CISS sequence8 was used. The CISS sequence is a heavily T₂-weighted gradient-echo sequence that allows reconstruction of slices in all planes with a reconstruction slice thickness of 0.7 mm. This sequence was used to determine the in vivo volume of the balloon and to estimate what proportion of the total intracranial volume the balloon comprised; this was accomplished by using a workstation and postprocessing software to obtain planimetric measurements in every slice. After deflation of the balloon, continuous measurements of structures on T₂-weighted and DW sequences were made over a 3-hour reperfusion period.

Statistical Analysis

In this paper balloon inflation time, the animal’s core temperature, SaO₂, and histological volumetric data are presented as absolute values and as means ± SDs. The intracranial volume of the balloon is given as the mean percentage ± SD. An unpaired t-test was used to compare the histologically determined size of the lesion in this study with that shown in Part I of our report.6 The Kolmogorov–Smirnov test (with the Lilliefors correction) was used to test data for normality. Significance was determined to be appropriate at a probability value lower than 0.05. Statistical analyses were performed using SigmaStat version 2.0.

Sources of Equipment and Supplies

The infusion pump used to inflate the epidural balloon was purchased from B. Braun (Melsungen, Germany). The Magneton Vi-
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Figure 1 upper left shows the baseline MR image with the fluid-filled balloon device lying over the left parietal cortex, which remains intact. One animal had to be excluded from this study because glue came in contact with the balloon and prevented its expansion. In the remaining 10 animals there was uniform expansion of the balloon (Fig. 1 upper right and lower left and right). Inflation of the balloon to 0.05 ml caused an extension of the draining tube system and did not result in balloon expansion. After inflation of the balloon to 0.1 ml there was a moderate mass effect with compression of the right lateral ventricle and a slight narrowing of the basal cisterns (Fig. 1 upper right). Inflation of the balloon to 0.2 ml (Fig. 1 lower left) resulted in an extensive space-occupying lesion with a midline shift and narrowing of the basal cisterns. Moreover, on T2-weighted sequences a white-matter region of hyperintensity, consistent with edema, developed predominantly in the right temporal lobe. After a completed balloon inflation to 0.3 ml (Fig. 1 lower right) there was a marked midline shift with compression of the basal cisterns. The volume of the balloon, as measured using the 3D CISS sequence (Fig. 2), was $0.3 \pm 0.04$ ml and the proportion of the total intracranial volume that the balloon comprised ranged from 5 to 7% (mean 6.1%, Table 1). In all animals the in vivo volume of the balloon did not change during a sustained inflation of 30 minutes. After 30 minutes of sustained inflation there was a marked progression in white-matter hyperintensity on T2-weighted images, especially in the right hemisphere, which could not be depicted on DW images (Fig. 3 left). Immediately after deflation of the balloon, we identified a slight cortical area of hyperintensity with a moderate mass effect in the left parietal lobe beneath the former area of balloon compression, which appeared bright on DW images (Fig. 3 right). The brain reexpanded to the calvaria and the morphological signs of raised intracranial pressure subsided with the reappearance of the basal cisterns and regression of midline displacement (Fig. 4 left). The bilateral white-matter edema markedly regressed after reperfusion. Within 3 hours after balloon deflation the hypertensive area in the left parietal cortex displayed a sharper demarcation and a regression of the mass effect (Fig. 4 right). Moreover, there was additional regression of the white-matter edema, which had been predominantly evident in the right temporal lobe. The narrowing of the basal cisterns had completely disappeared. The cerebrospinal fluid spaces almost completely reappeared with only a moderate residual midline shift. There were no relevant changes in physiological parameters throughout the entire experiment (Table 1).

Typical histopathological findings at the site of the well-demarcated lesion included thrombosed cortical vessels, cytotoxic edema, neuronal loss, and reactive astrocytes. Typically, the lesion extended caudally from the surface of the parietal lobe to the corpus callosum. In some cases tissue disruption and hemorrhage were present. The extension of the lesion on histopathological slices closely resembled the pathological appearance seen on MR images. The histopathological findings are described in detail and are accompanied by figures in Part I.

Histopathological morphometric analysis was performed by manual registration of the extent of tissue damage on cresyl violet–stained microscopic sections. The analysis did not show a significant difference between lesion areas in the animals at any stereotactic coordinates in Parts I and II of

**TABLE 1**

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Inflation Time (min)</th>
<th>Intracranial Core Temperature (°C)†</th>
<th>SaO2 (%)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>7.1</td>
<td>36.8 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>5.5</td>
<td>37 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>4.9</td>
<td>37.1 ± 0.3</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>5.8</td>
<td>37.1 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>6.4</td>
<td>37 ± 0.1</td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>5.1</td>
<td>36.8 ± 0.2</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>5.6</td>
<td>37.1 ± 0.1</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>6.5</td>
<td>37 ± 0.2</td>
</tr>
<tr>
<td>9</td>
<td>30</td>
<td>7.2</td>
<td>36.8 ± 0.4</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>6.8</td>
<td>37.2 ± 0.2</td>
</tr>
<tr>
<td>mean</td>
<td>30</td>
<td>6.1 ± 0.83†</td>
<td>37 ± 0.2</td>
</tr>
</tbody>
</table>

* Percentage of intracranial volume containing the expanded balloon.
† Values are expressed as the mean ± SD.
this combined investigation (p > 0.05). Summation of all lesion areas in Parts I and II showed a minor difference of 3.4%.

Discussion

Experimental investigations of traumatic injury have commonly applied the fluid-percussion, weight-drop, or cortical impact model,2–5,9,11,19 which have the advantage of providing reproducible and defined traumatic lesions; however, these models do not simulate the secondary brain damage that occurs following extraaxial traumatic lesions, which clinically often lead to delayed deficits especially after evacuation of an epidural or subdural hematoma. In the present study we have characterized a new epidural balloon-compression model in rats by using serial in vivo MR imaging. This model simulates the development and evacuation of an extraaxial hematoma and the sequel of secondary cytotoxic brain edema beneath the area of compression. Thus far a similar expansion model has only been evaluated in one study documenting secondary alterations after initiation of an epidural hematoma in four dogs over a 120-minute period.12 The rodent model described in Parts I and II of this study may facilitate further studies on brain damage following extraaxial mass lesions and may also be used to monitor therapeutic effects. The in vivo validation of the compression model has demonstrated a uniform expansion of the balloon and reproducible brain damage. The increased length of the liquid-filled polyethylene tube ended up in a growing “dead space” between the animals and the inflation pump, which had to be placed outside of the MR imaging device because it was ferromagnetic. Therefore, it was necessary to increase the flow rate slightly compared with that used in the experiments in Part I (0.6 ml/hour instead of 0.5 ml/hour, which was used in Part I experiments) to guarantee a comparable balloon volume for similar inflation periods in both studies. The flow rate required for the longer feeding tube was determined by the results of in vitro studies.

The form of anesthesia used in both parts of the study differed because of technical reasons. In Part II the focus was on an in vivo characterization of a new epidural balloon-
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compression model and not on any particular correlation between experimental data and MR imaging findings.

Serial MR imaging depicted characteristic changes on T_{2}-weighted and DW images during balloon inflation, de-compression, and reperfusion. In contrast to T_{2}-weighted sequences during balloon inflation, DW images did not reveal any increased signal in either temporal lobes; this indicated that there was a vasogenic edema at this stage. Immediately after reperfusion, both T_{2}-weighted and DW images revealed a bright signal in the area beneath the former balloon compression, which was consistent with cytotoxic edema, whereas the vasogenic component of the edema in both temporal lobes subsided during reperfusion.

Early changes on DW images have been reported in ves sel-occlusion models 6 to 60 minutes after onset of ischemia. They have been found to progress markedly within the first 2 hours after vessel occlusion.1,7,10,11,18 The earliest DW imaging–documented changes after traumatic brain injury were detected 30 minutes after onset of trauma. Using the weight-drop model, DW imaging revealed an opening in the blood–brain barrier and vasogenic edema within 30 to 60 minutes after trauma; this was followed by cytotoxic edema after 45 minutes, which increased to a maximum level after 7 to 14 days.1,3–5 Thereby, hypotension seems to prolong the vasogenic edema12 or induce a faster development of the cytotoxic component.11 In contrast with occlusion models in which an early cytotoxic component of edema develops10,16,18 all authors using closed-head injury,2,4 fluid-percussion,6 or cortical impact15 models have observed an early vasogenic component of edema overlapped by a cytotoxic component after 1 to 2 hours. With respect to the findings on edema in the literature, the balloon-expansion model presented in this study combines an early, short-lasting vasogenic component involving the temporal lobes with a cytotoxic component that develops immediately after reperfusion. Nevertheless, the vasogenic component of edema and the typical characteristics of an extraaxial lesion regressed after reperfusion until the end of the observation period.

A comparison of histological findings between both study groups (Part I and Part II) did not demonstrate any difference in the extension of the lesion, indicating a comparable amount of brain damage in both studies.

Conclusions

Magnetic resonance imaging confirmed the reproducibility of a novel balloon expansion model in rats. Whenever a new trauma model has to be validated, MR imaging should be incorporated as a supplementary tool to understand the underlying pathophysiology more fully.

References


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