Assessment of cerebral S100B levels by proton magnetic resonance spectroscopy after lateral fluid-percussion injury in the rat

ANDREA KLEINDENST, M.D., PH.D., CHRISTOS M. TOLIAS, M.D., PH.D., FRANK D. CORWIN, M.S., CHRISTIAN MÜLLER, PH.D., ANTHONY MARMAROU, PH.D., PANOS FATOUROS, PH.D., AND M. ROSS BULLOCK, M.D., PH.D.

Departments of Neurosurgery and Radiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia; and Institute of Laboratory Medicine and Pathobiocemistry, Charité, Humboldt University Berlin, Germany

Object. After traumatic brain injury (TBI), S100B protein is released by astrocytes. Furthermore, cerebrospinal fluid (CSF) and serum S100B levels have been correlated to outcome. Given that no data exist about the temporal profile of cerebral S100B levels following TBI and their correlation to serum levels, the authors examined whether proton magnetic resonance (MR) spectroscopy is capable of measuring S100B.

Methods. Results of in vitro proton MR spectroscopy experiments (2.35-tesla magnet, 25 G/cm, point-resolved spatially localized spectroscopy) revealed an S100B-specific peak at 4.5 ppm and confirmed a positive correlation between different S100B concentrations (10 nM–1 μM) and the area under the curve (AUC) for the S100B peak (r = 0.991, p < 0.001). Thereafter, proton MR spectroscopy was performed in male Sprague–Dawley rats (7 × 5 × 5-mm voxel in each hemisphere, TR 3000 msec, TE 30 msec, 256 acquisitions). Exogenously increased CSF S100B levels (~ 200 ng/ml) through the intraventricular infusion of S100B increased the AUC of the S100B peak from 0.06 ± 0.02 to 0.44 ± 0.06 (p < 0.05), whereas serum S100B levels remained normal. Two hours after lateral fluid-percussion injury, serum S100B levels increased to 0.61 ± 0.09 ng/ml (p < 0.01) and rapidly returned to normal levels, whereas the AUC of the S100B peak increased to 0.19 ± 0.04 at 2 hours postinjury and 0.41 ± 0.07 (p < 0.05) on Day 5 postinjury.

Conclusions. Proton MR spectroscopy proves a strong correlation between the AUC of the S100B peak and S100B concentrations. Following experimental TBI, serum S100B levels increased for only a very short period, whereas cerebral S100B levels were increased up to Day 5 postinjury. Given that experimental data indicate that S100B is actively released following TBI, proton MR spectroscopy may represent a new tool to identify increased cerebral S100B levels in patients after injury, thus allowing its biological function to be better understood.

Key Words: • proton magnetic resonance spectroscopy • S100B protein • traumatic brain injury • prognosis • rat

Measurements of the S100B protein as an early biochemical marker of the severity of brain damage is becoming more frequently performed in patients who have incurred TBI because serum S100B levels have been found to be a predictor of the consequent prognosis. Nonetheless, the correlation between high serum S100B levels and poor outcome following TBI seems to contradict the well-demonstrated beneficial effect of exogenously administered intraventricular S100B on cognitive function. The S100B protein belongs to a multigenic family of low molecular weight (9–13 kD) calcium-binding S100 proteins predominately abundant in astrocytes and constituting 1 to 1.5 μg/mg soluble protein in the brain. Experimental data demonstrate a neuroprotective and neurotrophic effect of the S100B protein, which is involved in signal transduction as well as regulation of enzyme activity and calcium homeostasis, stimulation of neurite outgrowth, and enhancement of cell maintenance. Note that S100B is actively secreted from astroglia and its release has been found to occur in cultured astrocytes within a few minutes after receptor activation, lasting for up to 10 hours. Data from additional investigations have confirmed the dose-dependent action of S100B, showing a beneficial effect on neurons at physiological nanomolar concentrations. In contrast, at micromolar concentrations, enhanced nitric oxide generation occurs, potentially leading to cell death. Many authors have reported that elevated serum levels of the S100B protein in head-injured patients correlate with a poor outcome. On the other hand, serum S100B levels are normally high directly after TBI, but become normalized by 24 hours postinjury in a high percentage of cases, even in those with a bad outcome. Results from another study in head-injured patients demonstrated a delayed increase in serum S100B levels after Day.

Abbreviations used in this paper: AUC = area under the curve; BBB = blood-brain barrier; CSF = cerebrospinal fluid; FPI = fluid-percussion injury; MR = magnetic resonance; NAA = N-acetyl-aspartate; PBS = phosphate-buffered saline; PRESS = point-resolved spatially localized spectroscopy; SEM = standard error of the mean; TBI = traumatic brain injury.
6 posttrauma, which was correlated to outcome, and attributed these findings to secondary brain cell damage. Whether elevated serum S100B levels following TBI or other brain insults are simply due to an increased passage from the central nervous system through a compromised BBB or are truly reflective of increased astrocytic production of S100B in response to trauma has yet to be investigated. Furthermore, there are no data about cerebral S100B levels and their correlation to serum and CSF levels. Because proton MR spectroscopy is an easily accessible tool for identifying cerebral metabolites like NAA, creatine, phosphocreatine, and choline, we attempted to determine whether MR spectroscopy is capable of noninvasively detecting increased cerebral S100B levels after TBI and to correlate these data to serum levels.

Materials and Methods

Animals and Surgical Procedure

The animal studies were conducted with the approval of the Institutional Animal Care and Use Committee and National Institutes of Health guidelines. Experiments were performed in 250- to 300-g adult male Sprague-Dawley rats. Surgery was performed after intubation with the rats placed in a state of isoflurane anesthesia and receiving controlled ventilation. A 4.9-mm craniotomy was trephined halfway between the lambda and bregma over the left hemisphere for FPI. Screws were inserted into the holes rostral and lateral to the craniotomy for stability, and a Luer-Loc hub made from the plastic end of a 20-gauge needle was cemented into the craniotomy by using dental cement. A fluid-percussion pulse of 2.09 ± 0.03 atm was administered through the craniotomy onto the intact dura mater by an FPI device. After the percussion pulse, the Luer-Loc hub was removed. Animals subjected to intraventricular infusion were placed in a stereotactic frame and fitted with a brain infusion cannula (3–5 mm, ALZET Corp., Cupertino, CA). The cannula was implanted according to the atlas of Paxinos and Watson with the tip aimed at the left lateral ventricle (stereotactic coordinates 0.8 mm behind the bregma, 1.5 mm lateral to the midline, 3–4 mm beneath the surface of the skull). Using dental cement, we secured the cannula to two stainless-steel screws inserted into holes made rostral and lateral. A microsomatic pump (Alzet model 1007D; DURECT Corp.) filled with a 90-μl infusion volume kept at 37°C was implanted subcutaneously in the neck and connected to the infusion cannula. After sutures were completed, anesthesia was discontinued and the animals were returned to the animal facility.

Proton MR Spectroscopy

Proton MR spectroscopy experiments were performed using a 2.35-tesla, 40-cm bore magnet (Bruker Instruments, Billerica, MA) equipped with an actively shielded gradient insert (12-cm inner diameter, maximum gradient strength 25 G/cm). Radiofrequency excitation/reception was performed using an actively decoupled radiofrequency coil set consisting of a birdcage design resonator (7-cm inner diameter) and a surface coil (2-cm diameter). Proton spectroscopy was performed using a water-suppressed spin echo voxel-localization technique (PRESS sequence). In Vivo Proton MR Spectroscopy Studies

The objective in these experiments was to assess whether proton MR spectroscopy is capable of detecting cerebral S100B levels increased by either exogenous intraventricular administration or endogenous release and to compare these levels with serum S100B levels. Therefore, animals were randomly assigned to the following groups: intraventricular vehicle (Group A, six rats) or S100B (Group B, six rats) infusion continuously for 5 days and subsequent MR spectroscopy, or lateral FPI and MR spectroscopy at 2 hours (Group C, eight rats) or on Day 5 (Group D, eight rats) postinjury. Purified bovine S100B protein (Calbiochem) was added to PBS containing 0.1 mg rat serum albumin/ml and was infused at a rate of 0.5 μl/hour (that is, 50 ng = 2.5-nM S100B infusion/hour). The microsomatic pumps were removed immediately before MR spectroscopy while the animal was in a state of pentobarbital anesthesia (30 mg/kg body weight). Blood samples for the measurement of S100B in serum were obtained at 2 hours posttrauma (Group A, five rats; Group C, five rats) or on Day 5 after trauma (Group B, 10 rats; Group D, 10 rats). After the experimental procedures were completed, all animals were killed using an overdose of pentobarbital.

Statistical Analysis

Commercially available software (SPSS, Inc., Chicago, IL) was used for statistical analysis. The levels of S100B in serum (nanograms per milliliter) as well as the AUC of the proton MR spectroscopy peak specific for S100B (4.6–4.3 ppm), choline (3.35–3.1 ppm), creatine (3.1–2.8 ppm), and ipsilateral NAA (2.2–1.8 ppm) were normalized to the contralateral NAA peak and analyzed using a randomized analysis of variance for group variations followed by a Tukey post hoc analysis. The correlation between the S100B concentration and the AUC of the peak on proton MR spectroscopy in the in vivo experiments was compared using a t-test. Statistical significance was set at a probability value less than 0.05.

Results

In Vivo Studies

The in vivo experiments demonstrated the feasibility of S100B detection on proton MR spectroscopy with a consistent peak at 4.5 ppm, which is close to the water peak. Normalization of the AUC of the S100B peak to the peak of the
Assessment of S100B on proton MR spectroscopy after FPI

10-nM S100B solution revealed 0.11 for the control PBS solution, 1.0 for the 10-nM S100B solution, 1.435 for the 100-nM S100B solution, and 1.939 for the 1-μM S100B solution (Fig. 1A), thereby demonstrating a significant correlation between the AUC measured on MR spectroscopy and the respective concentrations (r = 0.991, p < 0.01; Fig. 1B).

Effect of Intraventricular Infusion of S100B on MR Spectra and Serum Levels

No procedure in this study was related to death or infection, and all animals tolerated the surgical procedures and MR spectroscopy experiments well, without any obvious side effects. The proton MR spectroscopy examinations were performed within 30 to 60 minutes. Because the S100B peak was extremely close to the water peak, S100B measurements were rejected if water suppression in the PRESS sequence was inadequate. In three vehicle-infused animals no clear S100B peak was detectable, and in one S100B-infused animal the water suppression was insufficient; thus, the MR spectroscopy data in these animals were excluded from statistical analysis. The analysis of variance revealed a significant group effect for the MR spectroscopy assessment of the ipsilateral (F(3,18) = 5.195, p = 0.009) and contralateral (F(3,10) = 5.195, p = 0.030) S100B peak as well as the serum S100B data (F(3,50) = 3.135, p = 0.021). In uninjured animals intraventricularly infused with S100B, T2-weighted MR images demonstrated the track of the infusion needle (Fig. 2). The Tukey post hoc test for the AUC of the S100B peak normalized to the NAA peak indicated that intraventricular S100B infusion bilaterally increased the S100B peak significantly (Group B, five rats: ipsilateral 0.437 ± 0.065, p = 0.024; contralateral 0.545 ± 0.152, p = 0.025), compared with vehicle-infused animals (Group A, three rats, 0.060 ± 0.022). Measurement of serum S100B in vehicle-infused animals established normal levels at 0.062 ± 0.015 ng/ml, which were not altered after 5 days of intraventricular S100B infusion (0.079 ± 0.015 ng/ml, not significant).

Effect of TBI on S100B MR Spectra and Serum Levels

There was no injury-induced death among the rats. Water suppression was insufficient in four animals altogether, which were excluded from statistical analysis of the MR spectroscopy data. After lateral FPI, the T2-weighted MR image demonstrated no relevant edema or other intracranial pathology (Fig. 3). The Tukey post hoc tests revealed that the ipsilateral S100B peak tended to rise at 2 hours after trauma (Group C, six rats: 0.189 ± 0.039, p = 0.481) and significantly increased on Day 5 postinjury (Group D, six rats: 0.405 ± 0.073, p = 0.014), compared with control animals. A comparison of the AUC for the choline, creatine, and ipsilateral NAA MR spectroscopy peak revealed no intergroup differences (Table 1). The post hoc Tukey test of serum S100B indicated an early peak at 2 hours after injury (0.607 ± 0.036 ng/ml) compared with that in control animals (0.062 ± 0.015 ng/ml; p = 0.000) and normalized on Day 5 postinjury (0.067 ± 0.034 ng/ml, not significant; Fig. 4).

Discussion

There were three new findings in this study. First, proton MR spectroscopy—both in vitro and in vivo—can demonstrate the presence of the S100B protein, showing a significant correlation between the AUC of the S100B peak measured on MR spectroscopy and the respective S100B concentration. Thus, cerebral S100B levels exogenously increased by the intraventricular S100B infusion could be exhibited noninvasively for the first time. Second, proton MR
spectroscopy displayed increased endogenous S100B levels as soon as 2 hours after TBI and even up to Day 5 post-injury. Third, the cerebral S100B levels detected on proton MR spectroscopy do not correlate with the serum S100B levels measured at corresponding time points, thereby indicating that S100B release into the blood is modulated by additional factors, such as the integrity of the BBB, cerebral blood flow, and possibly degradation and sequestration by other organs.

**Feasibility of the Assessment of S100B on Proton MR Spectroscopy**

To identify whether proton MR spectroscopy was capa-

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**Fig. 2.** Proton MR spectroscopy (A) and MR imaging (B) results after intraventricular S100B infusion. A T_{2}-weighted MR image demonstrating the track of the infusion needle (B, arrow). A proton MR spectroscopy image exhibiting a S100B-specific peak at 4.5 ppm, which is not found in vehicle-infused animals (A, spectra of the ipsilateral side).

**Fig. 3.** Proton MR spectroscopy (A) and MR imaging (B) results after lateral FPI. On Day 5 after FPI, the T_{2}-weighted MR image revealed no relevant edema or other intracranial pathology. White boxes indicate the region of interest (RoI) from which the subsequent spectra were acquired. On proton MR spectroscopy, an S100B-specific peak at 4.5 ppm (close to the water peak) is depicted, although it cannot be found in association with vehicle-infused animals (spectra of the ipsilateral side).
Assessment of S100B on proton MR spectroscopy after FPI

### TABLE 1
Proton MR spectroscopy after intraventricular S100B infusion or lateral FPI

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Choline</th>
<th>Creatine</th>
<th>NAA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC</td>
<td>p Value†</td>
<td>AUC</td>
</tr>
<tr>
<td>ipsilateral side</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S100B infusion</td>
<td>0.060 ± 0.022</td>
<td>0.300 ± 0.035</td>
<td>0.387 ± 0.047</td>
</tr>
<tr>
<td>2 hrs postinjury</td>
<td>0.189 ± 0.039</td>
<td>NS</td>
<td>0.559 ± 0.038</td>
</tr>
<tr>
<td>5 days postinjury</td>
<td>0.405 ± 0.073</td>
<td>0.455 ± 0.054</td>
<td>0.559 ± 0.064</td>
</tr>
<tr>
<td>contralateral side</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S100B infusion</td>
<td>0.545 ± 0.152</td>
<td>0.507 ± 0.072</td>
<td>0.483 ± 0.064</td>
</tr>
<tr>
<td>2 hrs postinjury</td>
<td>0.200 ± 0.073</td>
<td>NS</td>
<td>0.623 ± 0.083</td>
</tr>
<tr>
<td>5 days postinjury</td>
<td>0.330 ± 0.077</td>
<td>0.462 ± 0.055</td>
<td>0.603 ± 0.088</td>
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<td>range (ppm)</td>
<td>4.6–4.3</td>
<td>3.35–3.1</td>
<td>3.1–2.8</td>
</tr>
</tbody>
</table>

* The AUC of the different peaks depicted on proton MR spectroscopy of the ipsilateral and contralateral side are normalized to the AUC of the ipsilateral NAA peak in control animals infused with vehicle and of the contralateral peak in the other groups, respectively. Compared with that for control animals infused with vehicle, the AUC of the S100B peak increased in both ipsilateral and contralateral sides after intraventricular S100B infusion (p < 0.05). Two hours after a lateral FPI, the AUC of the S100B peak started increasing and was on Day 5 after injury significantly higher than that for control animals (p < 0.05). The AUC of the choline, creatine, or ipsilateral NAA did not differ between the groups. Values represent the means ± SEM. Abbreviations: NS = not significant; — = not applicable.† Tukey post hoc analysis.

Assessment of Exogenously Administered S100B on Proton MR Spectroscopy

Because the underlying mechanism of S100B passage through the BBB has not been clarified and no data exist about cerebral S100B levels and their correlation to serum and CSF levels, we compared normal and exogenously increased cerebral S100B levels measured on MR spectroscopy with the respective serum levels in the context of an intact BBB. To increase the cerebral S100B concentrations we chose intraventricular infusion as the route of administration; in its transfer through the extracellular fluid, S100B has been found to affect hippocampal function. Furthermore, horseradish peroxidase, a protein with a molecular weight of 40 kD, is rapidly transferred from the CSF to the extracellular fluid of different brain regions. The 20-kD S100B protein is also supposed to be transferred.

For 5 days, we infused S100B at a concentration of 50 μg/ml and an infusion rate of 0.5 μl/hour (2.5 nM/hour) into the lateral ventricle. Assuming a CSF distribution volume of 250 μl and an hourly clearance of 25%, the CSF S100B concentration reached 200 ng/ml (10 nM). These levels are 100 times greater than normal (< 0.2 ng/ml in control volunteers to 0.8 ng/ml in older individuals) and exceeded by up to 10 times the upper serum levels that occur in traumatic pathologies. After 5 days of intraventricular S100B infusion, the S100B peak increased significantly in the hemisphere ipsilateral as well as the one contralateral to the infusion site, thereby verifying bilateral S100B distribution. Given that serum S100B levels remained normal, any relevant contribution of increased cerebral S100B levels to the respective blood concentrations appeared to be ruled out. These findings provided evidence that even high cerebral S100B levels may not pass through an intact BBB.

Analysis of S100B Release Into Serum After Experimental TBI

Analysis of serum S100B in our study revealed signif-
sicians and death agents, and as such, possibly represent the severity of a brain injury. For instance, the amount of intracerebral bleeding measured by CT is found to be strongly correlated to a worse outcome after TBI. The role of endogenously released S100B in the context of cerebral injury is mainly discussed in the following.

Assessment of Endogenously Released S100B on Proton MR Spectroscopy After Experimental TBI

After lateral FPI, the T2-weighted MR image demonstrated no relevant cerebral edema, whereas proton MR spectroscopy revealed an S100B peak as early as 2 hours after injury. On Day 5 postinjury, the S100B peak was even more increased and differed significantly from those in controls. Because neither the underlying mechanism of S100B passage through the BBB nor the relative contribution of astrocytic death, stimulated release, or BBB opening to serum levels has been clarified yet, we compared cerebral S100B levels measured on MR spectroscopy and the respective serum levels.

Given that the temporal profile of S100B serum levels—increasing immediately after FPI, peaking at 2 hours postinjury, and afterward promptly returning to normal values at 24 hours postinjury—parallels the BBB disruption occurring after experimental TBI, the passage of cerebral S100B into the blood seems to be a function mainly of BBB integrity. We could feasibly perform MR spectroscopy at only two time points after FPI, thereby not allowing the assessment of a complete profile of endogenous S100B release. Nevertheless, MR spectroscopy revealed slightly enhanced cerebral S100B levels 2 hours after FPI and significantly increasing levels thereafter to more than twice these values by Day 5 postinjury, thus supporting the hypothesis that S100B is actively secreted from astroglia. We and others have speculated that S100B is involved in an endogenous repair mechanism to overcome brain damage after injury. The other hand, the very rapid occurrence of high serum S100B levels immediately after injury may represent the contribution of intracellular S100B originating from damaged astrocytes and passing through an impaired BBB for a limited time.

A. Kleindienst, et al.

Implication of Experimental Findings on Assessment of Prognosis After TBI

The majority of experimental data demonstrate a neuroprotective and neurotrophic effect of the S100B protein, and S100B has been associated with synaptic remodeling, promotion of neural plasticity, and improved cognitive performance and memory function. Cognitive impairment is still one of the most disabling features following TBI and often longlasting behavioral sequelae occur, including decreased memory recall, slower processing of ideas, and increased irritability. A reliable method of estimating the endogenous restoration of these deficits would allow us to predict prognosis and thereby assist the treating physician in helping to protect the patient’s socioeconomic position and making better plans for the subsequent reintegration and rehabilitation processes. The assessment of cerebral S100B levels by using proton MR spectroscopy may thus offer a method of monitoring endogenous repair mechanisms essential for cognitive recovery after brain insults rather than merely demonstrate the extent of initial brain damage and consecutive BBB disruption, as serum S100B levels have been assumed to do.

Conclusions

Data from the current study—the first undertaken to demonstrate the feasibility of assessing cerebral S100B levels by performing proton MR spectroscopy—revealed increased endogenous S100B levelslasting up to Day 5 after experimental TBI. More importantly, we demonstrated that proton MR spectroscopy may represent an easily accessible tool for noninvasively identifying increased cerebral S100B levels and thus understanding its biology better. We therefore assert that proton MR spectroscopy may be a more reliable method of predicting prognosis after TBI than measuring serum S100B levels. Additional studies are needed to compare MR spectroscopy with outcome and other parameters, such as NAA levels.

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Assessment of S100B on proton MR spectroscopy after FPI

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