Cerebral edema formation is a crucial factor influencing the neurological outcome of patients who have experienced TBI. Any increase in brain water content augments ICP and reduces cerebral perfusion, possibly resulting in secondary damage related to ischemia. Two types of brain edema that develop after focal brain injury have been characterized. Vasogenic brain edema is caused by a disruption in the BBB, leading to an accumulation of plasma in the cerebral extracellular space. Disturbances in energetic and ionic homeostases result in excessive accumulation of intracellular water, known as cytotoxic edema formation. In both cases a net increase in cerebral water content will result in edema formation and volume expansion. Cellular edema formation, in turn, is reflected by increased extracellular levels of the volume regulator amino acid taurine and the ATP degradation products hypoxanthine and xanthine.

Because the primary damage caused by traumatic impact cannot be influenced therapeutically, it is important to determine what pharmacological agents can reduce evolving secondary damage. Secondary damage is related to compromises in functional and structural integrity in astrocytes, neurons, and endothelial cells, which among others are caused by glutamate-mediated excitotoxicity, lipid peroxidation, production of nitric oxide, formation of free radicals, disturbances in energetic and ionic homeostases, and activation of tissue and plasma mediators such as bradykinin. These changes are responsible for evolving posttraumatic vasogenic and cytotoxic brain edema formation and necrosis and are believed to explain secondary growth of contusion following focal TBI.

To date, the majority of neuroprotective drugs are directed toward attenuating cellular damage and reducing cytotoxic edema formation. Only a few drugs, such as aminosteroids and bradykinin B<sub>2</sub> receptor antagonists, have been used to attenuate BBB damage and reduce vasogenic brain edema formation. The B<sub>2</sub> receptor antagonists have been shown to attenuate brain damage and edema formation successfully following global and focal ischemia and cryogenically induced brain damage. Recently published results from a Phase II clinical study...
TABLE 1

Comparison of arterial blood gas measurements obtained at different time points in rats that received CCII*  

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean Physiological Parameters (range)</th>
<th>pH</th>
<th>PaCO₂, mm Hg</th>
<th>PaO₂, mm Hg</th>
<th>ABE, mmol/L</th>
<th>SBC, mmol/L</th>
<th>Hb, g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min before trauma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>7.42 ± 0.01 (7.22–7.5)</td>
<td>39.9</td>
<td>0.98 ± 0.09 (3.2–5.7)</td>
<td>24.4 ± 0.3 (21.8–25.7)</td>
<td>12.8 ± 0.2 (10–15.8)</td>
<td>11006</td>
<td></td>
</tr>
<tr>
<td>LF 16-0687Ms</td>
<td>7.40 ± 0.008 (7.34–7.47)</td>
<td>39.9</td>
<td>0.9 ± 0.007 (50.3–48.8)</td>
<td>24.4 ± 0.3 (21–25.7)</td>
<td>12.8 ± 0.2 (10–15.8)</td>
<td>11006</td>
<td></td>
</tr>
<tr>
<td>3 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>7.43 ± 0.008 (7.34–7.5)</td>
<td>39.9</td>
<td>0.9 ± 0.008 (50.3–48.8)</td>
<td>24.4 ± 0.3 (21.8–25.7)</td>
<td>12.8 ± 0.2 (10–15.8)</td>
<td>11006</td>
<td></td>
</tr>
<tr>
<td>LF 16-0687Ms</td>
<td>7.49 ± 0.008 (7.34–7.59)</td>
<td>39.9</td>
<td>0.9 ± 0.008 (7.34–7.59)</td>
<td>24.4 ± 0.3 (21.8–25.7)</td>
<td>12.8 ± 0.2 (10–15.8)</td>
<td>11006</td>
<td></td>
</tr>
<tr>
<td>30 mg/kg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>7.43 ± 0.001 (7.34–7.5)</td>
<td>39.9</td>
<td>0.9 ± 0.001 (3.2–5.7)</td>
<td>24.4 ± 0.3 (21.8–25.7)</td>
<td>12.8 ± 0.2 (10–15.8)</td>
<td>11006</td>
<td></td>
</tr>
<tr>
<td>LF 16-0687Ms</td>
<td>7.49 ± 0.008 (7.34–7.59)</td>
<td>39.9</td>
<td>0.9 ± 0.008 (7.34–7.59)</td>
<td>24.4 ± 0.3 (21.8–25.7)</td>
<td>12.8 ± 0.2 (10–15.8)</td>
<td>11006</td>
<td></td>
</tr>
</tbody>
</table>

* ABE = acid–base exchange; Hb = hemoglobin; SBC = standard bicarbonate.

and a multicenter, randomized, placebo-controlled clinical trial9 in patients with TBI revealed protective effects. The observed neuroprotection is thought to be mediated by the inhibition of constitutive B₂ receptors 10 located on the luminal and abluminal membranes of cerebral endothelial cells, on smooth-muscle cells of cerebral arteries and postcapillary venules, 11 and on astrocytes 12 and neurons. 13 Consequently, administration of a B₂ receptor antagonist should counteract the action of the endogenous B₂ receptor agonist bradykinin. Bradykinin is a potent mediator of vasogenic brain edema formation 14,15 because it causes arteriolar dilation, venous constriction, 16 and increased BBB permeability. 17,18,19 A novel nonpeptide kinin B₂ receptor antagonist, LF 16-0687Ms, potently binds to the human B₂ receptor, providing an inhibition constant of 0.67 nM. 20 In the present study the protective potential of LF 16-0687Ms was evaluated in a rat model of focal TBI. In addition, changes in the CSF amino acids taurine and glutamate and the ATP degradation products hypoxanthine and xanthine, known to be associated with cellular edema formation, 21,22,23 were studied in a subset of 10 rats.

Materials and Methods

Animal Population

Forty male Sprague–Dawley rats weighing 250 to 350 g each (Charles River, Deutschland GmbH, Sulzfeld, Germany) were used in the experiments. The animals were given approximately 24 hours to become accustomed to the laboratory before the study was performed. The experimental protocol was approved by the committee for animal research in Berlin, Germany.

Anesthesia Induction and Maintenance

Anesthesia was induced in all animals and maintained with isoflurane and N₂O/O₂. After induction (isoflurane 5 vol%, N₂O 0.5 L/min, O₂ 0.3 L/min), anesthesia was tapered (isoflurane 1–2.5 vol%, N₂O 0.5 L/min, O₂ 0.3 L/min) to maintain stable MABP values between 80 and 90 mm Hg. The animals continued to breathe spontaneously. Anesthesia was maintained during surgery, trauma, and drug administration, and throughout the first 20 minutes following drug administration. Anesthesia was again induced 24 hours after trauma to allow recording of MABP, ICP, CPP, blood sampling, and brain removal. Intracranial pressure was measured with a Codman ICP microsensor (Johnson & Johnson Medical Ltd., Berkshire, UK).

Surgery and Trauma

Within 10 minutes after anesthesia was induced, an arterial catheter was implanted in the right femoral artery and MABP was recorded throughout the study. The rats were positioned in a stereotactic holder and a left parietotemporal craniotomy was made within the scalp. The dura mater was stripped off the underlying brain, and brain removal. Intracranial pressure was measured with a Codman ICP microsensor (Johnson & Johnson Medical Ltd., Berkshire, UK).

Drug Dosage and Administration

The LF 16-0687Ms was given in either a low (3 mg/kg body weight) or high (30 mg/kg body weight) dose and was dissolved in physiological saline 10 minutes before administration. One milliliter of LF 16-0687Ms solution containing a low (3 mg/kg) or high (30 mg/kg) dose was injected subcutaneously 5 minutes after trau-
Bradykinin and traumatic brain edema

There were no differences between groups.

**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MABP, mm Hg (range)</th>
<th>ICP, mm Hg (range)</th>
<th>CPP, mm Hg (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>Before Trauma</td>
<td>84 ± 1 (77–93)</td>
<td>1 (77–93)</td>
</tr>
<tr>
<td>LF 16-0687Ms</td>
<td>3 mg/kg</td>
<td>87 ± 1 (77–105)</td>
<td>2 (72–105)</td>
</tr>
<tr>
<td></td>
<td>30 mg/kg</td>
<td>84 ± 2 (74–100)</td>
<td>2 (75–102)</td>
</tr>
</tbody>
</table>

**Results**

**Arterial Blood Gas Levels**

Arterial blood gas levels were within the same ranges in all animals at all time points: 10 minutes before trauma, 25 minutes after drug administration, and before brain removal (Table 1). There were no differences between groups.

**Changes in MABP, ICP, CPP, and Rectal Temperature**

The mean arterial blood pressure remained within narrow limits at all time points in all animals. There was no difference between different groups at any investigated time point (Table 2). Administration of LF 16-0687Ms did not result in any adverse effects on MABP, which remained stable. At the time point of brain removal, ICP and CPP in all animals were within the same range (Table 2). There were no significant differences between groups. Rectal temperature was maintained between 37°C and 38°C in all animals at all investigated time points. There was no difference between the groups.

**Brain Swelling**

Swelling of the traumatized hemisphere was significantly decreased in animals that received the low and high dose of LF 16-0687Ms subcutaneously, compared with control animals (Fig. 1). The decrease was 25% in animals that received the low dose and 27% in those that received the high dose. Thus administration of a high dose of LF 16-0687Ms did not decrease brain swelling any further than a low dose.

**Hemispheric Water Content**

Twenty-four hours after brain trauma and drug admin-
istration, the water content of the traumatized hemisphere was significantly increased compared with that of the non-traumatized hemisphere (Fig. 2). In rats receiving the low or high dose of LF 16-0687Ms, there was less water content in the traumatized hemisphere than in control rats. In the nontraumatized hemisphere water content tended to be increased in rats that received the low or high dose of LF 16-0687Ms compared with control animals (Fig. 2). There was no difference between low- and high-dose groups.

Calculating the arithmetic difference in water content between traumatized and nontraumatized hemispheres demonstrated a significant reduction in hemispheric water content in rats treated with the low or high dose of LF 16-0687Ms, compared with control rats (Fig. 3).

Changes in Cisternal CSF Levels of Taurine, Glutamate, Hypoxanthine, and Xanthine

In CSF, the taurine, glutamate, hypoxanthine, and xanthine levels were significantly increased in traumatized rats compared with nontraumatized animals (p < 0.0051). Results in nontraumatized animals were collected from the works of Kornhuber, et al.,19 and Eells, et al.4 (Table 3).

The low dose of LF 16-0687Ms significantly decreased the CSF levels of the volume regulator amino acid taurine and the ATP degradation products hypoxanthine and xanthine, compared with levels found in traumatized control rats receiving physiological saline (p < 0.05). The CSF glutamate levels were significantly increased in treated rats (p < 0.005) (Table 3).

Discussion

Posttraumatic subcutaneous administration of a single dose of the nonpeptide kinin B, receptor antagonist LF 16-0687Ms successfully reduced the formation of brain edema following CCII in rats. This is reflected by significantly reduced brain swelling and decreased cisternal CSF taurine, hypoxanthine, and xanthine levels. These results are consistent with those of a previous study in which it was demonstrated that LF 16-0687Ms markedly reduced cerebral edema in a rat model of closed head trauma.28 Administration of LF 16-0687Ms appears safe because the animals’ MABP and arterial blood gas levels were not influenced.

Kallikrein–Kinin System and Brain Edema

The physiological role of the kallikrein–kinin system, which is widely distributed within the central nervous system remains unclear. The kallikrein–kinin system is believed to “process precursors of neuronal cell growth factors that maintain the integrity of transmitters and synaptic function.”3 Under normal conditions, activation of the kallikrein–kinin system is tightly regulated because precursor proteins (tissue and plasma kininogens) and specific tissue and plasma enzymes (kallikreins) are only present in their inactive forms,3 requiring enzymatic cleavage to enable activation of these enzymes with the subsequent production of kinins such as bradykinin. Furthermore, spontaneous activation of this system is prevented by a variety of endogenous inhibitors, such as the Cl-esterase inhibitor, α1-macroglobulin, α1-antitrypsin, and antithrombin, demonstrating an interrelationship among blood clotting, complement, and kinin activation.34 Under normal circumstances, bradykinin is only present in very low concentrations and its very short biological half life (< 30 seconds)3 is explained by fast deactivation through specific enzymes (kininas).3

Under pathological conditions, however, the role of the kallikrein–kinin system has been characterized more closely. Both traumatic and ischemic brain damage are associated with the activation of the kallikrein–kinin system, resulting in the sustained production of bradykinin as kallikreins mediate cleavage of kinins such as bradykinin.
Bradykinin and traumatic brain edema

from precursor tissue and plasma kininogens. It is widely accepted that the actions of released bradykinin are mainly mediated by the activation of constitutive B2 receptors. These bradykinin receptors are widely distributed within the central nervous system and are present on the luminal and abluminal side of endothelial cells, on smooth-muscle cells of cerebral arterioles and postcapillary venules, on astrocytes, and on neurons. Activation of these G protein–coupled B2 receptors results in activation of intracellular second messenger systems, leading to increased uptake and mobilization of calcium, sustained activation of phospholipases A2 and C, and increased production of nitric oxide. 

Activation of these second messenger systems, in turn, results in the production of prostaglandins and leukotrienes and is associated with intracellular release of calcium. These endogenous substances are well-known mediators of cell damage.

Bradykinin has been shown to cause and aggravate vasogenic brain edema formation, which is mediated by a breakdown in the BBB, arteriolar vasodilation, and venous constriction. This leads to sustained extravasation of proteins and plasma into brain tissue, the hallmark of vasogenic edema formation. Furthermore, it is believed that the activation of the second messenger systems mediates and aggravates cytotoxic edema formation. In this respect, bradykinin has been shown to release excitatory amino acids such as glutamate and aspartate from astrocytes.

Reduction of Brain Edema Formation With B2 Receptor Antagonists

Successful reduction of brain edema formation by blocking B2 receptors underlines the contribution of the kallikrein–kinin system in the pathophysiology of traumatic brain damage. At present, the exact targets addressed by the administered B2 receptor antagonist in the present model of focal contusional lesion cannot be defined. It remains to be answered whether there are decreases in BBB damage, in the number of necrotic cells, and cellular edema formation, as well as ameliorated perfusion within the vicinity of the contusion. Histological and functional analyses are warranted to address these different potential targets, which are important denominators in influencing secondary growth of cerebral traumatic lesions.

As early as 90 minutes following CCII, both the formation of cytotoxic and that of vasogenic brain edema formation have been shown to develop, as determined by magnetic resonance imaging. Although cytotoxic edema formation continues to increase during the first 24 hours following trauma, vasogenic edema formation has been shown to decrease over time, after demonstrating maximum Evans blue dye extravasation between 4 and 6 hours after CCII. Based on these results, BBB damage and the resulting vasogenic edema formation mainly occur in the early phase following CCII. Therefore, BBB damage and related activation of the kallikrein–kinin system appear to be potential targets for the specific B2 receptor antagonist LF 16-0687Ms, administered early after trauma.

As reflected by the significantly decreased levels of the volume regulator amino acid taurine and the ATP degradation products hypoxanthine and xanthine, edema formation appears to be attenuated in rats that receive the B2 receptor antagonist. To counteract a pathological increase in cell volume, taurine is released nonvesicularly. As taurine leaves swollen cells, it shifts ions and water from the intracellular to the extracellular compartment, restoring initial cell volume. Traumatic brain injury is associated with edema formation and increased CSF taurine levels, as observed in severely brain injured patients and in the rats in the present investigation. Therefore, decreased CSF taurine levels could reflect a reduction in cellular swelling, as observed in LF 16-0687Ms–treated rats.

Brain edema formation and degradation of energy substrates (ATP) show a strong interrelationship. A decrease in ATP is associated with a substantial increase in cerebral water content. Adenosine triphosphate is essential in stabilizing membrane potential and transport processes. Any lack in energy substrates will result in a breakdown of membrane potential and transport processes. Among other consequences, ionic homeostasis is impaired, which results in accumulation of intracellular water and sustained glutamate toxicity. Because increased hypoxanthine and xanthine levels reflect ATP degradation, it is conceivable that energy impairment is present in the investigated brain-injured rats. The significant decrease in CSF levels of hypoxanthine and xanthine could, therefore, reflect attenuated ATP degradation and edema formation in those rats treated with the B2 receptor antagonist LF 16-0687Ms.

At present it remains unclear why cerebral water content tends to be higher in the nontraumatized hemisphere of rats treated with the B2 receptor antagonist LF 16-0687Ms. In a closed head trauma model, LF 16-0687Ms tended to reduce and not increase the water content of the uninjured hemisphere. Injury to the contralateral hemisphere can be excluded because it appears normal in histological studies. Furthermore, 24 hours after trauma, the BBB of the contralateral hemisphere remains intact, as assessed by extravasation of Evans blue dye. Systemic explanations, such as hypoxemia and insufficient cerebral

---

**TABLE 3**

Measurements of cisternal CSF concentrations of substances associated with cellular edema formation in nontraumatized control animals and in traumatized rats receiving NaCl or LF 16-0687Ms*

<table>
<thead>
<tr>
<th>Substance (μM)</th>
<th>Nontraumatized Animals</th>
<th>Control Group (5 animals)</th>
<th>LF 16-0687Ms–Treated Group (5 animals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>taurine</td>
<td>14.58 ± 1.47†</td>
<td>62.4 ± 2.9§</td>
<td>43.8 ± 1.6§</td>
</tr>
<tr>
<td>glutamate</td>
<td>2.56 ± 0.17†</td>
<td>14.7 ± 0.9§</td>
<td>28.6 ± 8.6§</td>
</tr>
<tr>
<td>hypoxanthine</td>
<td>2.8 ± 0.2</td>
<td></td>
<td>7.6 ± 0.8§</td>
</tr>
<tr>
<td>xanthine</td>
<td>2.0 ± 0.3</td>
<td></td>
<td>105.3 ± 8.4§</td>
</tr>
</tbody>
</table>

* The traumatized control group received NaCl. The LF 16-0687Ms–treated group received a drug dose of 3 mg/kg body weight.
† Data from nontraumatized animals were collected from the study of Kornhuber, et al. (nine animals).
‡ p < 0.005 compared with nontraumatized animals.
§ p < 0.005 compared with traumatized control animals.
|| Data from nontraumatized animals were collected from the study of Eells, et al. (eight animals).
perfusion, seem unlikely because all animals survived and did not have any abnormal blood pressure or arterial blood gas values 20 minutes after drug administration and before brain removal (24 hours after injury). Most likely, administration of LF 16-0687Ms leads to a nonspecific increase in cerebral water content by influencing normal regulatory mechanisms.

The significantly increased glutamate levels found in the CSF in rats that received LF 16-0687Ms compared with control rats could eventually contribute to the increased water content of the nontraumatized hemisphere. Following TBI, release of glutamate results in pathologically elevated levels in the extracellular space. Decreased clearance and continuing synaptic and nonvesicular release could account for the persistently elevated CSF glutamate levels. Theoretically, LF 16-0687Ms could interfere with these uptake and release mechanisms. Direct intracerebral application of bradykinin has been reported to decrease synaptic activity. Because glutamate mediates the synaptic excitation, blocking bradykinin-mediated synaptic silencing with LF 16-0687Ms could thus result in sustained synaptic activity. Convulsions, however, were not observed in these rats. At present, it remains unclear whether the observed increase in CSF glutamate levels is only transient and whether these elevated glutamate concentrations induce further brain damage at longer survival periods.

Additional detailed investigations in which electroencephalographic, histological, laser Doppler, and microdialysis studies are used are required to elucidate the exact mechanisms of the B2 receptor antagonist LF 16-0687Ms following TBI.

Acknowledgment

The technical assistance provided by Prof. Dr. Kempski in the HPLC analysis is gratefully acknowledged.

References

Bradykinin and traumatic brain edema


Manuscript received July 12, 1999. Accepted in final form January 15, 2000.
Address reprint requests to: John F. Stover, M.D., Department of Neurosurgery, Charité–Virchow Medical Center, Augstenburger Platz 01, D-13353 Berlin, Germany. email: john.stover@charite.de.