Background. The pathogenesis and mechanisms of septic encephalopathy are not completely understood. We compared two different models of sepsis: lipopolysaccharide-induced endotoxemia and cecal ligation and puncture (CLP) bacteremia in rats with respect to changes in endothelial expression of the adhesion molecules intercellular adhesion molecule-1 (ICAM-1), platelet-endothelial cell adhesion molecule-1 (PECAM-1), and of cerebral albumin extravasation as a marker for capillary breakdown of the blood brain barrier.

Material and methods. Male Wistar rats were divided into control, endotoxemia, or CLP-group. Mean arterial blood pressure was measured via femoral artery catheterization. Brain tissue for immunohistochemistry was harvested at 1 h, 6 h, and 24 h after induction of sepsis.

Results. The CLP-group showed a decrease in mean arterial pressure after 24 h in comparison with the sham-group ($P < 0.05$). Cerebral ICAM-1 expression was at its maximum 24 h after induction of sepsis, with the highest expression in the CLP-group. There was no difference in PECAM-1 expression between the groups. Cerebral albumin extravasation increased early after 6 h in both septic groups with a maximum at 24 h after induction of sepsis.

Conclusion. These results suggest that there are early changes in the integrity of the blood-brain barrier in the central nervous system in an ongoing septic progress. This provides evidence that these changes are due to inflammatory mediators, and not to the presence of live bacteria. Increased ICAM-1 expression might be an early factor involved in these pathogenic events. Although the role of PECAM-1 cannot conclusively be determined, we were able to show its expression on cerebral endothelium in all groups.

Key Words: septic encephalopathy; albumin; blood brain barrier; ICAM-1; PECAM-1.

INTRODUCTION

Septic encephalopathy is a multifactorial and frequent complication in severe sepsis. Although it is predominant in the intensive care unit, a common classification is difficult.

The incidence of septic encephalopathy varies between 9% and 100%, depending on the different studies and definitions [1–5]. There is agreement that the clinical appearance of septic encephalopathy is an acute, reversible reduction of cerebral functions during a septic progress, which is associated with a higher mortality rate [5, 6]. Its onset can be before or after the occurrence of general signs of sepsis [4, 5].

There is evidence in the literature that clinical scores, such as the Glasgow-Coma-Scale, are good predictive clinical markers for the mortality rate of the ongoing central septic process [1, 7].

The multifactorial pathogenesis and underlying mechanisms of the septic encephalopathy are not completely understood. Endotoxin or other bacterial products activate macrophages and lymphocytes, which contribute to neuronal damage [8–10]. Oxidative stress and enhanced production of cytokines (interleukin [IL]-1β; IL-2; tumor necrosis factor-alpha [TNF-α]) seem to have a neurotoxic effect on the brain [4, 5] or are involved in neuronal apoptosis [11].
We know that in addition to cytokines, enhanced expression of cell adhesion molecules (CAMs) such as intercellular adhesion molecule-1 (ICAM-1), E-selectin, or platelet-endothelial cell adhesion molecule-1 (PECAM-1) play an important role in the pathophysiology of sepsis.

Cytokine-induced selectin expression mediates the contact of leukocytes with the blood vessel wall [12]. After activation of leukocytes by chemokines and chemo-attractants (e.g., interleukin-8) firm adhesion of the cells to the vessel wall occurs. This step is mediated via interaction of cell adhesion molecules (e.g., ICAM-1) and the surface expression of selectins on leukocytes. Transmigration of the adherent cells through the junctions of endothelium is mediated by PECAM-1 [13]. However, little data exists for these changes in the brain during ongoing septic encephalopathy.

Two different models of sepsis in rats were used in this study. Endotoxemia (lipopolysaccharide [LPS]) and bacteremia (cecal ligation and puncture [CLP]) with respect to the induced cerebral changes in the endothelial expression of the adhesion molecules ICAM-1 (CD54) and PECAM-1 (CD31) were compared. In addition, we analyzed the time course of cerebral albumin extravasation as a marker for capillary breakdown of the blood brain barrier.

**MATERIALS AND METHODS**

All experiments were performed in accordance with the guidelines for research with experimental animals and were approved by the Governmental Animal Protection Committee. Adult male Wistar rats (250 to 300 g body weight) were housed in cages in a temperature-controlled animal room. Free access to food and water was given throughout the experimental period. The rats were randomized into four groups with seven animals each.

**Septicemia Protocols**

Adult male Wistar rats, weighing 250 to 300 g, were subjected to the control (sham-group), endotoxemia (LPS), or bacteremia (CLP) model. All rats were anesthetized with halothane (0.5 to 1.5 vol% in a mixture of nitrous oxide (60%) and oxygen (40%) using face masks. The right internal jugular vein was cannulated with a polyethylene catheter.

For the endotoxemia model, rats were subjected to 3 mg/kg LPS from *Escherichia coli* (LPS from *Escherichia coli* 026:B6; Sigma Chemicals, Deisenhofen, Germany) dissolved in 0.9% NaCl with respect to the induced cerebral changes in the endothelial expression of the adhesion molecules ICAM-1 (CD54) and PECAM-1 (CD31) were compared. In addition, we analyzed the time course of cerebral albumin extravasation as a marker for capillary breakdown of the blood brain barrier.

Alterations in MAP are common during ongoing sepsis. After induction of sepsis, MAP stayed stable in the control (sham-group), with changes being significant 24 h after cecal ligation and puncture (86 ± 22 mm Hg versus 126 ± 18 mm Hg; mean ± SD; P < 0.05). Differences resulting in MAP are common during ongoing sepsis. After induction of sepsis, MAP stayed stable in the LPS-group at all measured time points (Table 1, Fig. 1). The CLP-group showed a continuous decrease in MAP in comparison with the sham-group, with changes becoming significant 24 h after cecal ligation and puncture (86 ± 22 mm Hg versus 126 ± 18 mm Hg; mean ± SD; P < 0.05).

**Blood Gas Analysis**

The base excess was significantly decreased after 6 h in both groups representing sepsis. After an initial decrease in pH in both groups of sepsis, pH values in the LPS-group returned back to normal after 24 h (Table 1). No significant differences in hematocrit were found between groups (Table 1).
Two common models of sepsis are LPS-induced inflammation and cecal ligation and puncture, initially characterized by Wichterman and colleagues in 1980 [15]. The LPS model is easy to perform and induces most clinical changes, which can also be found in septic through LPS or CLP, we detected an early increase in cerebral albumin extravasation in the both groups. The LPS-group (2.07 ± 0.12) and the CLP-group (2.27 ± 0.12) showed maximum albumin extravasation 24 h after induction of sepsis (sham-group 0.1 ± 0.14) (Table 4; Fig. 1), indicating a damage of the blood-brain-barrier.

**DISCUSSION**

By using two different animal models of sepsis the present study demonstrates an increasing ICAM-1 expression over time and a continuous expression of PECAM-1 on vascular endothelium compared with sham operated rats. Albumin extravasation into brain tissue points out changes in the integrity of the blood brain barrier.

No significant differences between the groups were found for the expression of PECAM-1 on endothelium of cerebral vessels (Table 2).

**TABLE 2**

ICAM-1 Expression After 1, 6, and 24 Hours (h) After Induction of Sepsis for the Different Groups: Control (Con), Sham Operated (Sham), Endotoxin (LPS), Cecal Ligation and Puncture (CLP) (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>1 h</th>
<th>6 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>0 ± 0</td>
<td>0.2 ± 0</td>
<td>0.3 ± 0.14</td>
</tr>
<tr>
<td>Sham</td>
<td>0.2 ± 0</td>
<td>0.2 ± 0</td>
<td>1 ± 0.2*</td>
</tr>
<tr>
<td>LPS</td>
<td>0.4 ± 0</td>
<td>0.87 ± 0.12</td>
<td>1.27 ± 0.12*</td>
</tr>
<tr>
<td>CLP</td>
<td>0.27 ± 0.12</td>
<td>0.93 ± 0.12</td>
<td>1.27 ± 0.12*</td>
</tr>
</tbody>
</table>

* P < 0.05.

**Immunohistochemistry**

ICAM-1 endothelial surface expression in the LPS- and CLP-group increased continuously over the experimental time period. Both groups representing sepsis had their maximum ICAM-1 expression 24 h after the induction of sepsis. The highest values were measured in the CLP-group compared with the sham-group (1.27 ± 0.12 versus 0.3 ± 0.14; mean ± SD; P < 0.05) (Table 2; Fig. 2).

By using two different animal models of sepsis the present study demonstrates an increasing ICAM-1 expression over time and a continuous expression of PECAM-1 on vascular endothelium compared with sham operated rats. Albumin extravasation into brain tissue points out changes in the integrity of the blood brain barrier.
We found that there was an enhanced expression of ICAM-1 in brain tissue vessels soon after induction of sepsis [6; 24 h] in both the LPS- and the CLP-group. This finding agrees with a study by Henninger et al. [22], who showed that 5 h after administration of endotoxin (LPS) a TNF-α mediated ICAM-1 expression starts, with a maximum after 24 h. Bohatschek and colleagues [23] show a disruption of the blood-brain barrier after systemic injection of LPS in a mouse model with induction of ICAM-1, granulocyte influx, and albumin extravasation. Furthermore, transgenic deletion of ICAM-1 reduced the number of infiltrating granulocytes by more than 50%. A different study also indicated the important role of ICAM-1 in LPS-induced septic shock [24]. ICAM-1 deficient mice were resistant to otherwise effective doses of endotoxin and showed decreased neutrophil infiltration and endothelial damage in the liver. Using two well-established models of sepsis, our data provide evidence for enhanced ICAM-1 expression in the brain at very early stages after induction of sepsis. This enables enhanced cerebral leukocyte/endothelial interaction through cytokines, causing alterations in the blood-brain barrier, followed by brain edema and neuronal cell death [25]. The results of Weigand et al. [10] show that elevated levels of circulating ICAM-1 in septic shock are associated with the development of liver dysfunction. ICAM-1 modulation leading to firm leukocyte adhesion and transmigration is described as an important step in sepsis-induced tissue damage. Derived from a rat model, there is evidence that ICAM-1 expression in ischemic brain microvessels may contribute to enhanced leukocyte adherence and persistent activation [26].

**Cerebral ICAM-1 expression in our models of sepsis is due to inflammatory mediators and not to the presence of live bacteria, because we did not find differences between the CLP- and the LPS-group.**

**PECAM-1**

PECAM-1 is an important part of the intercellular junction within the endothelium and has an essential role for the transmigration of leukocytes [27, 28]. There is little data concerning the role of PECAM-1 in the pathophysiology of septic encephalopathy. Blocking of PECAM-1 with monoclonal antibodies has been described to inhibit transmigration of neutrophils and monocytes [29]. A study of Qing et al. [30] showed that during inflammation antigenic specific T-cell migration in the central nervous system (CNS) is mediated by PECAM-1. In contrast, PECAM-1 deficient mice are more vulnerable to systemic LPS administration and show an increased inflammatory response [31]. Our results show an unchanged PECAM-1 expression before and after LPS stimulation in all groups, which is in accordance with former studies [22]. In addition, we used a second model of sepsis with live bacteria to confirm these findings. There were no differences in PECAM-1 expression before and after induction of sepsis detected.

**Albumin**

We found significantly elevated albumin extravasation into cerebral tissue for both models of sepsis, being

### TABLE 3

**PECAM-1 Expression After 1, 6, and 24 Hours (h) After Induction of Sepsis for the Different Groups: Control (Con), Sham Operated (Sham), Endotoxin (LPS), Cecal Ligation and Puncture (CLP) (mean ± SD)**

<table>
<thead>
<tr>
<th></th>
<th>1h</th>
<th>6h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>2.7 ± 0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>2.5 ± 0.14</td>
<td>2.8 ± 0.28</td>
<td>2.7 ± 0.14</td>
</tr>
<tr>
<td>LPS</td>
<td>2.73 ± 0.31</td>
<td>2.73 ± 0.31</td>
<td>2.8 ± 0.27</td>
</tr>
<tr>
<td>CLP</td>
<td>2.93 ± 0.12</td>
<td>2.93 ± 0.12</td>
<td>2.73 ± 0.31</td>
</tr>
</tbody>
</table>

### TABLE 4

**Albumin Extravasation 1, 6, and 24 Hours (h) After Induction of Sepsis for the Different Groups: Control (Con), Sham Operated (Sham), Endotoxin (LPS), Cecal Ligation and Puncture (CLP) (mean ± SD)**

<table>
<thead>
<tr>
<th></th>
<th>1h</th>
<th>6h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>0 ± 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>0 ± 0</td>
<td>0.2 ± 0</td>
<td>0.1 ± 0.14</td>
</tr>
<tr>
<td>LPS</td>
<td>1 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>2.07 ± 0.12*</td>
</tr>
<tr>
<td>CLP</td>
<td>0.73 ± 0.12</td>
<td>2 ± 0.2</td>
<td>2.27 ± 0.12*</td>
</tr>
</tbody>
</table>

*P < 0.05.
most predominant in the CLP-group. This result corresponds to other publications, describing albumin extravasation in toxin-induced rats already after 1 h with a maximum after 24 h after induction of sepsis [32]. Albumin extravasation correlates with cerebral tissue changes after cerebral infarction in rats [33] and seems to be a marker for damage in the central nervous system. In a model of experimental meningitis TNF-α being a key cytokine of the septic pathway is associated with the extravasation of albumin across the blood-brain barrier into the CNS [34]. The results of Bohatschek and colleagues [23] show a disruption of the blood-brain barrier with albumin extravasation and induction of ICAM-1 after systemic injection of LPS in a mouse model. Our findings are in accordance with the results of Barichello et al. [35]. They describe oxidative stress triggering cerebral damage in rats at a very early phase of septic encephalopathy, which was induced by cecal ligation and puncture. Assuming that an intact blood-brain barrier does not allow albumin extravasation, we conclude that there are alterations on cerebral vascular endothelium soon after the onset of sepsis. In conclusion, we demonstrate in two animal models of sepsis that there are early changes in the integrity of the blood-brain barrier in the CNS. We provide data that these changes are due to inflammatory mediators and not to the presence of live bacteria. Considering the course of time ICAM-1 might be a factor causing these pathogenetic events. We could not detect differences in PECAM-1 expression between the groups in either model, leaving its role in sepsis and septic encephalopathy undetermined.

REFERENCES


