Hepatic Effects of Thoracic Epidural Analgesia in Experimental Severe Acute Pancreatitis


Background: Thoracic epidural anesthesia (TEA) protects the intestinal microcirculation and improves perioperative outcomes. TEA also reduces mortality in acute experimental pancreatitis. Its impact on hepatic microcirculation, however, in health and critical illness is unknown. Therefore, the authors studied the effect of TEA on the liver in healthy rats and in experimental severe acute pancreatitis.

Methods: TEA was induced by 15 μl/h bupivacaine, 0.5%. Necrotizing pancreatitis was induced by intraductal infusion of 2 ml/kg taurocholic acid, 5%. Twenty-eight rats were assigned to either Sham operation, Sham + TEA, Pancreatitis, or Pancreatitis + TEA. After 15 h, mean arterial pressure, heart rate, and respiratory function were recorded. Sinusoidal width and perfusion rate and the intrahepatic leukocyte adhesion were assessed by intravital microscopy. In an additional 22 rats randomly assigned to Sham, Pancreatitis, and Pancreatitis + TEA, hepatic apoptosis was evaluated by staining for single-stranded DNA and Fas ligand–positive cells.

Results: TEA did not affect hepatic microcirculation and leukocyte adhesion in healthy rats. Blood pressure remained unchanged in the Sham + TEA group. In Pancreatitis, mean arterial pressure decreased from 141 ± 6 mmHg to 127 ± 13 mmHg but remained stable in Pancreatitis + TEA. The sinusoidal diameter decreased from 5.4 ± 0.1 μm to 5.0 ± 0.2 μm in Pancreatitis. This was restored in Pancreatitis + TEA. Intrahepatic leukocyte adhesion was not affected by TEA. The increased hepatocyte apoptosis in Pancreatitis was abolished in Pancreatitis + TEA. This might be mediated by inhibition of the Fas ligand pathway.

Conclusion: TEA reduces liver injury in necrotizing acute pancreatitis. This could be related to a regional sympathetic block. TEA could thus preserve liver function in systemic inflammatory disorders such as acute pancreatitis.

THORACIC epidural anesthesia (TEA) allows superior pain therapy after abdominal and thoracic surgery and may reduce postoperative mortality. It has been established as a cornerstone in the multimodal perioperative care after major surgery.

The liver is critically involved in a multitude of physiologic processes and contributes decisively to the host immune reaction in injury, sepsis, and inflammation. Preserved liver function is crucial to maintain homeostasis in the perioperative period and in critical illness.

Perioperatively, however, liver function is impaired and hepatocellular damage occurs. In liver resections, liver injury occurs before resection only because of liver manipulation. In rat liver transplantation, hepatic manipulation halves the graft survival rate. During laparoscopic cholecystectomy, low-flow ischemia seems to contribute to hepatic dysfunction.

In critical illness, hepatic dysfunction is related to a poor prognosis. In a mixed intensive care unit patient population, hepatic dysfunction early after admission increased mortality by 80%. In severe sepsis and trauma, liver injury was associated with increasing mortality and duration of hospital stay. The hepatic immune response determines pathogen clearance and the systemic immune reaction. After prolonged inflammatory reactions, both hepatic and systemic immune dysfunction contribute to mortality.

Sympathetic nerve activity plays a crucial role in hepatic injury and immune response. Stressful social encounter induces liver injury in male mice. In animal studies, autonomic denervation of the liver reduced perioperative hepatic injury. In sepsis, α and β adrenoceptors influence hepatocellular dysfunction and immune response. Sympathetic activity also affects regeneration after liver resections.

Sympathetic block is thought to be a key mechanism of the protective effects of TEA. Intestinal effects have been extensively investigated in clinical and animal studies. In contrast to this, the knowledge about hepatic effects of TEA is limited. Until today, the influence of TEA on hepatic microcirculation has not been investigated.

Moreover, the effects of TEA on hepatic microvascular injury and leukocyte adhesion in critical illness are unknown.

Therefore, we conducted a randomized, blinded study to test the hypothesis that (1) TEA influences hepatic microvascular perfusion and leukocyte activation in health and (2) TEA reduces hepatic microvascular disturbance, inflammation, and apoptosis in critical illness induced by severe acute pancreatitis in rats.

Materials and Methods

The study was approved by the animal ethics committee of the district government of Muenster, Germany. Animals
received standard chow and were kept in a 12 h light–dark cycle. Food was withheld 12 h before surgery.

Male Sprague-Dawley rats (275–300 g; Harlan-Winkelmann, Borchen, Germany) were anesthetized by isoflurane in 50% oxygen. Central venous and arterial lines (0.96 mm OD; Liquidscan, Ueberlingen, Germany) were inserted. Epidural catheters (0.61 mm OD) were inserted at L3–L4 and advanced to T6.23 All catheters were exteriorized at the neck of the animal and protected by a swivel device (TSE-Systems, Bad Homburg, Germany).

After midline laparotomy, the duodenum was carefully lifted and placed with slight tension on the ventral face of the transverse colon. By this, the head of the pancreas was exposed and the common bile duct could be visualized in a straight line. The proximal bile duct was temporarily clamped. Acute pancreatitis was induced by retrograde intraductal injection of 2 ml/kg taurocholate, 5%.36 The rats were then allowed to wake up. Epidural infusion was commenced immediately after surgery. Saline, 2 ml/h intravenously, was infused throughout the experiment.

All animals were randomly assigned by closed envelopes to four groups: Sham—sham procedure, 15 μl/h NaCl, 0.9%, epidural; Sham + TEA—sham procedure, 15 μl/h bupivacaine, 0.5%, epidural; Pancreatitis—acute pancreatitis, 15 μl/h NaCl, 0.9%, epidural; or Pancreatitis + TEA—acute pancreatitis, 15 μl/h bupivacaine, 0.5%, epidural. The investigators were blinded as they were not aware of the group assignment. All measurements were performed 15 h after induction of acute pancreatitis. At this time, pancreatitis was fully developed but there still was no mortality.32

After 15 h, mean arterial blood pressure was recorded by a standard pressure transducer (PMSET 1DT; Becton Dickinson, Heidelberg, Germany) and a monitor (Siemens Sirecust 404; Siemens, München, Germany). Heart rate was recorded using the arterial pressure curve. Eighty microliters full blood was withdrawn for blood gas analysis. Muscular tone and function were quantified separately in sinusoids and postsinusoidal venules. Temporarily adherent leukocytes, i.e., cells stagnant at the sinusoidal wall for less than 20 s, and permanently adherent leukocytes, i.e., stagnant for more than 20 s, were counted in each acinus and expressed as cells/μm². Accordingly, temporarily and permanently adherent leukocytes in the venules were counted as cells/μm² venular endothelium.

Liver Injury and Apoptosis
An additional 22 animals were randomly assigned to the Sham group (n = 8), Pancreatitis group (n = 7), and Pancreatitis + TEA group (n = 7) to assess hepatic apoptosis, Fas ligand expression, and serum transaminase activity. One blood sample in the Sham group was lost as a result of technical difficulties after sampling. Therefore, one additional Sham animal was included in the randomization process, resulting in 8 tissue samples in the sham group.

After 15 h, blood was withdrawn via aortic puncture and was centrifuged at 4°C for 10 min at 3,000g immediately after sample collection. Plasma was stored at −80°C until use. Plasma enzyme activity of aspartate aminotransferase and alanine aminotransferase was determined by means of standard enzymatic techniques (n = 7 in each group) (Ektachem; Kodak, Stuttgart, Germany). Specimens of the left liver lobe were collected immediately after death and fixed by immersion in 4% formaldehyde solution. Subsequently, they were dehydrated and embedded in paraffin wax to cut sections at a thickness of 5 μm.

All histologic and immunohistochemical investigations were performed by an experienced pathologist unaware

Intravital Microscopy
Twenty-eight animals (n = 7 each group) were reanesthetized and tracheotomized 15 h after pancreatitis induction or sham procedure. Intravital microscopy of the left liver lobe was performed as follows: Median laparotomy was extended by a left subcostal incision after thorough coagulation of left epigastric vessels. Then, the hepatic ligaments of the left liver lobe were carefully dissected. The animal was placed in a 110° position on its left side onto the microscope (Eclipse 300; Nikon, Düsseldorf, Germany). The left liver lobe was exteriorized without distortion. The lower surface was placed on a coverslip in a tension free position. Sodium fluorescein (Sigma, Deisenhofen, Germany), 2 μmol/kg body weight, was used to enhance contrast between blood plasma in the sinusoidal lumen and liver tissue. Rhodamine 6G (Sigma), 0.2 μmol/kg body weight, was used for leukocyte staining. The temperature of the preparation was kept normothermic by warm saline solution.

In each experiment, 10 randomly chosen acini and 10 postsinusoidal venules were recorded for 30 s with both sodium fluorescein and rhodamine contrast enhancement. The images were videotaped and evaluated by a blinded investigator off-line. Image analysis was performed using a computer-assisted image analysis system (AnalySIS; Olympus Soft Imaging Systems, Muenster, Germany).

For assessment of hepatic microvascular perfusion, the following variables were measured: (1) perportal sinusoidal diameter (in micrometers) of 10 sinusoids per acinus and (2) number of nonperfused sinusoids divided by all visible sinusoids of the acinus.

Leukocyte–endothelial cell interaction was evaluated separately in sinusoids and postsinusoidal venules. Temporar}

of group assignment. Immunostaining on paraffin sections for single-strand DNA was performed as follows. After antigen retrieval (Revia; Biocarta, Hamburg, Germany) for 5 min in a domestic pressure cooker and after blocking nonspecific binding sites with bovine serum albumin-c basic blocking solution (1:10 in phosphate-buffered saline; Aurion, Wageningen, The Netherlands), the sections were immunoreacted with the primary antibodies (1:1,500, rabbit polyclonal; IBL, Gunma, Japan) overnight at 4°C, and after washing in phosphate-buffered saline, the sections were treated for 10 min with methanol containing 0.6% hydrogen peroxide to quench endogenous peroxidase. For fluorescent visualization of bound primary rabbit antibodies, sections were further treated for 1 h at room temperature with DAKO anti-rabbit EnVision horseradish peroxidase (DAKO Deutschland, Hamburg, Germany). The horseradish peroxidase label was amplified with fluorescein isothiocyanate–conjugated tyramine at a dilution of 1:300 in phosphate-buffered saline in the presence of 0.02% H2O2 for 10 min. Samples were counterstained for 15 s with 4',6-diamidino-2-phenylindole (5 µg/ml; Sigma) and mounted with Vectashield (Vector Laboratories, Burlingame, CA).

Fas ligand was stained after antigen retrieval by immunoreacting 25 min with the primary antibodies (1 µg/ml, goat polyclonal; R&D-Systems, Wiesbaden, Germany) at room temperature. A commercially available secondary visualization system (DAKO labeled streptavidin–alkaline phosphatase) was used. Counterstaining was performed by modified hematoxylin staining, and slides were mounted with Xylol (Sigma)–Pertex (Medite, Burgdorf, Germany).

Apoptosis was assessed separately for hepatocytes and nonparenchymal cells. The apoptosis index, i.e., the proportion of single-strand DNA decorated nuclei per 100 nuclei, was semiquantitatively by counting approximately 1,000 cells in seven randomly chosen fields of view, containing approximately 110 hepatocytes and 35 nonparenchymal cells each. The presence of Fas ligand in liver sections was nonquantitatively assessed as positive or negative for each animal. One set of sections was stained with hematoxylin–eosin and assessed semiquantitatively with respect to incidence and extent of portal and acinar inflammation, edema, and necrosis.

**Statistical Analysis**

Sigmapstat 3.0 (Systat Software, Richmond, CA) was used for statistical analysis. Data were tested for normality and equal variance. The effects of TEA on healthy liver microcirculation and leukocyte adhesion were evaluated by comparison of the Sham group and the Sham + TEA group using a two-tailed t test. Pancreatitis-induced and TEA-related effects were evaluated by comparing Sham, Pancreatitis, and Pancreatitis + TEA groups by one-way-analysis of variance with the *post hoc* Bonferroni test. The incidence of Fas ligand positivity was compared by Fisher exact test. Group size resulted from an *a priori* sample size estimation performed with Sigmapstat 3.0 based on the estimated differences of means and SDs of parameters of hepatic microcirculation derived from previous studies of the authors and existing literature. The following assumptions were made: analysis of variance in three groups, power 0.5; *P* < 0.05; sinusoidal width: ∆mean 0.5 µm, SD 0.25 µm; loss of sinusoidal perfusion: ∆mean 9.5%, SD 5%. Data are presented as mean and 95% confidence interval. Motor deficits are displayed as median [25%–75%). Statistical significance was defined as *P* < 0.05.

**Results**

All animals survived the observational period. In the TEA groups, mild motor deficits of the hind limbs occurred with a median motor score of 0 [0–1]. In Sham + TEA, mean arterial pressure and heart rate did not change. In Pancreatitis, mean arterial pressure was decreased compared with Sham, whereas heart rate remained stable. TEA did not induce further hemodynamic deterioration in the Pancreatitis + TEA group. Arterial oxygen tension, arterial carbon dioxide tension, and pH remained constant in all groups (table 1). Catheter placement was verified by autopsy in each animal. The median catheter tip position was T6 [T5–T7].

**Table 1. Cardiorespiratory Measurements**

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Sham + TEA</th>
<th>Pancreatitis</th>
<th>Pancreatitis + TEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mmHg</td>
<td>141 (135–147)</td>
<td>139 (135–143)</td>
<td>127 (114–140)*</td>
<td>133 (127–139)</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>401 (369–433)</td>
<td>428 (402–454)</td>
<td>411 (358–464)</td>
<td>447 (411–483)</td>
</tr>
<tr>
<td>PaO2, mbar</td>
<td>92 (87–97)</td>
<td>95 (88–102)</td>
<td>91 (71–111)</td>
<td>103 (93–113)</td>
</tr>
<tr>
<td>PacO2, mbar</td>
<td>35.6 (32.8–38.4)</td>
<td>35.4 (30.5–40.3)</td>
<td>36.8 (32.4–41.0)</td>
<td>35.4 (33.8–37.0)</td>
</tr>
<tr>
<td>pH</td>
<td>7.44 (7.42–7.46)</td>
<td>7.43 (7.41–7.45)</td>
<td>7.38 (7.29–7.47)</td>
<td>7.42 (7.40–7.44)</td>
</tr>
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</table>

Hemodynamic and respiratory parameters 15 h after induction of acute pancreatitis and sham procedure, respectively. There were no significant effects of epidural anesthesia in healthy animals. Untreated acute pancreatitis with continuous volume replacement induced a mild hypotension that was not further aggravated by epidural anesthesia. All other parameters were not affected by untreated acute pancreatitis or acute pancreatitis with epidural anesthesia.

* *P* = 0.03 vs. Sham; all data are displayed as mean [95% confidence interval]; *n* = 7 in each group.

MAP = mean arterial pressure; PaCO2 = arterial carbon dioxide tension; PaO2 = arterial oxygen tension; TEA = thoracic epidural anesthesia.
Table 2. Effects of TEA on Hepatic Microcirculation in Healthy Rats

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Sham + TEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinusoidal diameter, μm</td>
<td>5.4 (5.3–5.5)</td>
<td>5.2 (4.9–5.5)</td>
</tr>
<tr>
<td>Nonperfused sinusoids, %</td>
<td>2.8 (0.3–5.1)</td>
<td>3.5 (1.9–5.1)</td>
</tr>
<tr>
<td>Sinusoidal rollers, 1/mm²</td>
<td>116 (0–241)</td>
<td>231 (0–538)</td>
</tr>
<tr>
<td>Sinusoidal stickers, 1/mm²</td>
<td>885 (0–1,770)</td>
<td>1,473 (844–2,500)</td>
</tr>
<tr>
<td>Venular rollers, 1/mm²</td>
<td>38 (0–91)</td>
<td>31 (7–55)</td>
</tr>
<tr>
<td>Venular stickers, 1/mm²</td>
<td>135 (0–343)</td>
<td>73 (8–138)</td>
</tr>
</tbody>
</table>

In healthy animals, there were no significant effects of epidural anesthesia on hepatic microcirculation and the number of temporarily, i.e., less than 20 s (“rollers”), and permanently, i.e., more than 20 s (“stickers”), adherent leukocytes 15 h after sham procedure. All data are displayed as mean (95% confidence interval); n = 7 in each group.

TEA = thoracic epidural anesthesia.

Effects of TEA on Hepatic Microcirculation in Healthy Rats

Compared with Sham, hepatic sinusoidal perfusion was not altered in Sham + TEA. The sinusoidal width was not influenced, and the sinusoidal perfusion rate remained constant. Neither the sinusoidal nor the post-sinusoidal, venular leukocyte endothelial cell interaction was influenced in Sham + TEA (table 2).

Effects of TEA on Hepatic Microcirculation in Acute Pancreatitis

In Pancreatitis, sinusoidal vasoconstriction (P = 0.022 vs. Sham) and significant increase in nonperfused sinusoids (P = 0.002 vs. Sham) were induced. Treatment by TEA prevented sinusoidal constriction (P = 0.015 vs. Pancreatitis), whereas sinusoidal perfusion did not change in Pancreatitis + TEA compared with Pancreatitis (fig. 1). Neither temporal nor permanent sinusoidal adhesion was affected in Pancreatitis or in Pancreatitis + TEA. The venular leukocyte endothelial cell interaction also did not change in Pancreatitis and Pancreatitis + TEA (table 3).

Effects of TEA on Liver Cell Apoptosis and Injury in Acute Pancreatitis

Neither in Pancreatitis nor in Pancreatitis + TEA were serum activities of alanine aminotransferase and aspartate aminotransferase significantly influenced (data not shown). Pancreatitis induced mild portal and hepatic acinar inflammation, hepatic edema, and formation of tissue necrosis that was not affected by treatment (data not shown).

In Pancreatitis, a significant increase in overall apoptotic cells was recorded (P = 0.025 vs. Sham; fig. 2). This was mainly due to increased hepatocyte apoptosis (P = 0.008 vs. Sham). TEA reduced overall apoptosis in Pancreatitis + TEA (P = 0.032 vs. Pancreatitis). The reduction was driven by reduced hepatocyte apoptosis (P = 0.008 vs. Pancreatitis). Changes in nonparenchymal cell apoptosis did not reach the level of significance in the Pancreatitis and Pancreatitis + TEA groups (fig. 2).

In Pancreatitis, expression of Fas ligand was recorded in four of seven specimens compared with zero of eight in Sham (P = 0.026 vs. Sham; fig. 3). In Pancreatitis + TEA, one of seven liver sections was positive for Fas ligand (not significant vs. Pancreatitis).

Table 3. Effects of TEA on Intrahepatic Leukocyte Adherence in Acute Pancreatitis

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Pancreatitis</th>
<th>Pancreatitis + TEA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinusoidal rollers, 1/mm²</td>
<td>116 (0–241)</td>
<td>281 (19–543)</td>
<td>303 (18–588)</td>
</tr>
<tr>
<td>Sinusoidal stickers, 1/mm²</td>
<td>885 (0–1,770)</td>
<td>2,576 (1,405–3,747)</td>
<td>2,659 (339–4,979)</td>
</tr>
<tr>
<td>Venular rollers, 1/mm²</td>
<td>38 (0–91)</td>
<td>65 (3–127)</td>
<td>118 (16–220)</td>
</tr>
<tr>
<td>Venular stickers, 1/mm²</td>
<td>135 (0–343)</td>
<td>80 (22–138)</td>
<td>128 (75–181)</td>
</tr>
</tbody>
</table>

There were no significant effects of epidural anesthesia on the number of temporarily, i.e., less than 20 s (“rollers”), and permanently, i.e., more than 20 s (“stickers”), adherent leukocytes 15 h after induction of pancreatitis or sham procedure. All data are displayed as mean (95% confidence interval); n = 7 in each group.

TEA = thoracic epidural anesthesia.
Discussion

This study is the first to demonstrate the effects of TEA on hepatic microcirculation in healthy and critically ill rats. In healthy rats, no changes occurred in sinusoidal perfusion and intrahepatic leukocyte–endothelial cell interaction. In acute pancreatitis, however, TEA was able to prevent sinusoidal vasoconstriction. The pattern of leukocyte adhesion was not affected. TEA reduced apoptotic cell death after acute pancreatitis.

Effects of TEA on Hepatic Microcirculation in Healthy Rats

In contrast to the intestinal findings of earlier studies, the hepatic microperfusion was not affected by continuous TEA in the current study. The total regional blood flow to the liver is not affected by TEA in instrumented pigs because of a constant portal venous blood flow that compensates the mild decrease in hepatic arterial blood flow. Hepatic oxygen delivery and tissue oxygenation remain constant during TEA in instrumented anesthetized pigs and dogs.

Hepatic perfusion is subject to complex regulatory processes to which both sympathetic and parasympathetic activity contribute. It is perfectly adjusted to maintain metabolic homeostasis, an adapted systemic release of proteins, substrates, and mediators, and to mobilize and retain intravascular blood volume.
Sympathetic and parasympathetic regulation of liver blood flow occurs both at presinusoidal and postsinusoidal sphincters. Under resting conditions in health, there is little tonic sympathetic activity, whereas vagal nerve activity tonically influences hepatic blood flow. Hepatic denervation did not change resting blood flow in pigs but only impaired hepatic buffer response during reduced portal inflow.

Consequently, in the current study the unchanged hepatic microcirculation during TEA might be explained by the low tonic sympathetic activity in the liver in healthy rats. Furthermore, sympathetic block due to general anesthesia might conceal the effects of TEA. Recently, a mouse model has been described that allows determination of hepatic blood flow in awake and unrestrained animals. This model might be suitable to determine regional hepatic blood flow during TEA. However, liver intravital microscopy inevitably requires anesthetized animals.

**Hepatic Effects of TEA in Acute Pancreatitis**

In liver injury induced by systemic inflammation such as sepsis and acute pancreatitis, a multitude of mediators and microvascular injury interact. This includes activation of hepatocytes, Kupffer cells, and neutrophil granulocytes.

**Microcirculation.** Sinusoidal vasoconstriction and loss of sinusoidal perfusion occur early in systemic inflammation and contribute to organ injury. Inflammation and impaired perfusion are associated with cellular hypoxia. Increased release of vasoconstrictors such as endothelin and thromboxane on the one side associated with decreased or insufficiently increased release of vasodilators such as nitric oxide and carbon monoxide on the other side constitutes a sinusoidal vasoconstrictive state. This is aggravated by sinusoidal and stellate cell swelling. In the current study, TEA prevented sinusoidal vasoconstriction, whereas sinusoidal loss was not influenced.

In contrast to the resting condition, in the face of increased sympathetic tone, hepatic microcirculation and cell injury are significantly affected. In healthy livers, electrical stimulation of the hepatic sympathetic nerves induces a strong decrease in hepatic blood flow. Stimulants of sympathetic activity such as psychic stress in adult male mice, baroreceptor response, acute urinary retention, or painful stimuli during anesthesia reduce hepatic regional blood flow. In a pig model of liver surgery and manipulation, hepatic denervation exerted differential effect in living compared with brain-dead pigs, possibly related to altered sympathetic activity.

Hence, sympathetic block by TEA might have mediated the decreased vasoconstrictive response in severe acute pancreatitis, whereas no such response was recorded in the healthy liver. Hepatic stellate cells and sinusoidal endothelial cells are important regulators of sinusoidal diameters. Under norepinephrine stimulation, isolated stellate cells contract and release proinflammatory cytokines in both α1 and β receptor-dependent pathways. Both stellate cells and epithelial cells can be activated by α-adrenergic stimulation.

Because regulation of sinusoidal perfusion also occurs at both presinusoidal and postsinusoidal sites, the exact site of regulation cannot be identified in this study.

**Leukocyte Adhesion.** Neutrophil and Kupffer cell immune reactions including the production of extracellular reactive oxygen intermediates, protease release, and chemotaxis are a hallmark in primary and secondary hepatic injury. Inagaki et al. described increased permanent leukocyte adherence after acute edematous pancreatitis that was related to increased cellular injury.

In this study, neither temporary nor permanent leukocyte adhesion to sinusoidal wall and venular endothelium was affected by pancreatitis or TEA. In an earlier study in severe acute pancreatitis, increased sinusoidal leukocyte rolling was demonstrated. The differing results might be explained by a time effect. In the current study, measurement was performed after 15 h, whereas leukocyte rolling was found to be increased only after 6 h and, to a lesser extent, 12 h, with normal values after 24 h. This observation is also in accord with findings of a time-dependent pattern of leukocyte recruitment after cecal ligation and puncture-induced sepsis in rats with increased leukocyte adhesion after 7 h and normalized values after 20 h. In a recent study, accordingly, hepatic neutrophil recruitment started to decline after 8 h. In earlier periods of severe acute pancreatitis, in which leukocyte recruitment is increased, TEA might exert more pronounced effects.

**Apoptotic Cell Death.** Apoptosis occurs in numerous extrapancreatic tissues and immune cell populations during acute pancreatitis. Hepatocyte apoptosis in acute pancreatitis is partly induced by FasL secretion by Kupffer cells. However, Kupffer cells also undergo apoptosis during acute pancreatitis, thereby influencing systemic immune competence. In models of hepatic ischemia–reperfusion and portal endotoxin challenge, Fas and FasL are also supposed to contribute to hepatic apoptosis. Both sympathetic and parasympathetic activities affect the regulation of intrahepatic apoptosis and liver regeneration.

However, experimental data are partially conflicting. On the one hand, Yu et al. have demonstrated hepatic apoptosis in a state of increased sympathetic activity induced by acute urinary retention. Hepatic denervation reduced apoptotic cell death in this model. On the other hand, overexpression of β2 adrenoceptors, norepinephrine application, and β-receptor stimulation reduced hepatocyte apoptosis in mice, rats, and hepatocyte cultures, respectively. Furthermore, acute hepatic
failure caused by Fas/Fas ligand-induced apoptosis can be reduced by hepatic denervation.44

In the current study, pancreatitis-induced increase of apoptotic cell death was prevented by TEA. Hepatic FasL expression increased in acute pancreatitis, and after treatment with TEA a trend toward reduction of FasL positivity occurred. The applied model of TEA in rats has been shown to be related to thoracodorsal sympathetic block.25 These data suggest that sympathetic block can counteract the increased apoptosis in critical illness and that this effect may be mediated by the Fas/Fas ligand pathway. However, further studies are needed to elucidate the role of sympathetic regulation of apoptosis in critical illness.

Summary

Impaired liver function is a severe complication in the postoperative course and critical illness. Strategies to prevent or treat liver failure might improve morbidity and mortality in these patients. This study demonstrated that TEA did not influence hepatic microcirculation in healthy rats. However, TEA reduced the effects of acute pancreatitis on microcirculation and apoptotic cell death. In rats, intralobular sympathetic innervation is lower compared with other animals and human liver.56 These results suggest that TEA might protect the liver in the perioperative period and in critical illness.

The authors thank Christina Großerichter (Laboratory Technician, Department of Anesthesiology and Intensive Care Medicine, University Hospital Muenster, Muenster, Germany) for her expert technical assistance.

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