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Hepatic Effects of Thoracic Epidural Analgesia in Experimental Severe Acute Pancreatitis

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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.[1–3] It has been established as a cornerstone in the multimodal perioperative care after major surgery.[3,4]

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The liver is critically involved in a multitude of physiologic processes and contributes decisively to the host immune reaction in injury, sepsis, and inflammation.[5–7] 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.[8] In rat liver transplantation, hepatic manipulation halves the graft survival rate.[9] During laparoscopic cholecystectomy, low-flow ischemia seems to contribute to hepatic dysfunction.[10]

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%.[11] In severe sepsis and trauma, liver injury was associated with increasing mortality and duration of hospital stay.[12,13] The hepatic immune response determines pathogen clearance and the systemic immune reaction.[5,14] After prolonged inflammatory reactions, both hepatic and systemic immune dysfunction contribute to mortality.[15,16]

Sympathetic nerve activity plays a crucial role in hepatic injury and immune response. Stressful social encounter induces liver injury in male mice.[17] In animal studies, autonomic denervation of the liver reduced perioperative hepatic injury.[9,18] In sepsis, α and β adrenoreceptors influence hepatocellular dysfunction and immune response.[19–21] Sympathetic activity also affects regeneration after liver resections.[22]

Sympathetic block is thought to be a key mechanism of the protective effects of TEA.[23–27] Intestinal effects have been extensively investigated in clinical and animal studies.[28–33] In contrast to this, the knowledge about hepatic effects of TEA is limited.[34,35] 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 introduced. 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 using an established motor score.[30] The categories of this score are as follows: 0 = normal tone, free movement of the hind limbs; −1 = weak hypotonia of the hind limbs and body posture; −2 = moderate hypotonia of the hind limbs and body posture; and −3 = inability to support the body on the hind limbs and flat body posture.

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 coverglass 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) periportal 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. Temporary 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/μm^2. Accordingly, temporarily and permanently adherent leukocytes in the venules were counted as cells/μm^2 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 3,000*g* 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

of group assignment. Immunostaining on paraffin sections for single-strand DNA was performed as follows. After antigen retrieval (Revial; 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% H_2O_2 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 biotin–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 semiquantitated 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

Sigmastat 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 Sigmastat 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: Δmean 0.5 μm, SD 0.25 μm; loss of sinusoidal perfusion: Δmean 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

	Sham	Sham + TEA	Pancreatitis	Pancreatitis + TEA
MAP, mmHg	141 (135–147)	139 (135–143)	127 (114–140)*	133 (127–139)
Heart rate, beats/min	401 (369–433)	428 (402–454)	411 (358–464)	447 (411–483)
Pa_{O_2}, mbar	92 (87–97)	95 (88–102)	91 (71–111)	103 (93–113)
Pa_{CO_2}, mbar	35.6 (32.8–38.4)	35.4 (30.5–40.3)	36.8 (32.4–41.0)	35.4 (33.8–37.0)
pH	7.44 (7.42–7.46)	7.43 (7.41–7.45)	7.38 (7.29–7.47)	7.42 (7.40–7.44)

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; Pa_{CO_2} = arterial carbon dioxide tension; Pa_{O_2} = arterial oxygen tension; TEA = thoracic epidural anesthesia.

Table 2. Effects of TEA on Hepatic Microcirculation in Healthy Rats

	Sham	Sham + TEA
Sinusoidal diameter, μm	5.4 (5.3–5.5)	5.2 (4.9–5.5)
Nonperfused sinusoids, %	2.8 (0.5–5.1)	3.5 (1.9–5.1)
Sinusoidal rollers, 1/mm^2	116 (0–241)	231 (0–538)
Sinusoidal stickers, 1/mm^2	885 (0–1,770)	1,473 (844–2,500)
Venular rollers, 1/mm^2	38 (0–91)	31 (7–55)
Venular stickers, 1/mm^2	135 (0–343)	73 (8–138)

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 postsinusoidal, 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).

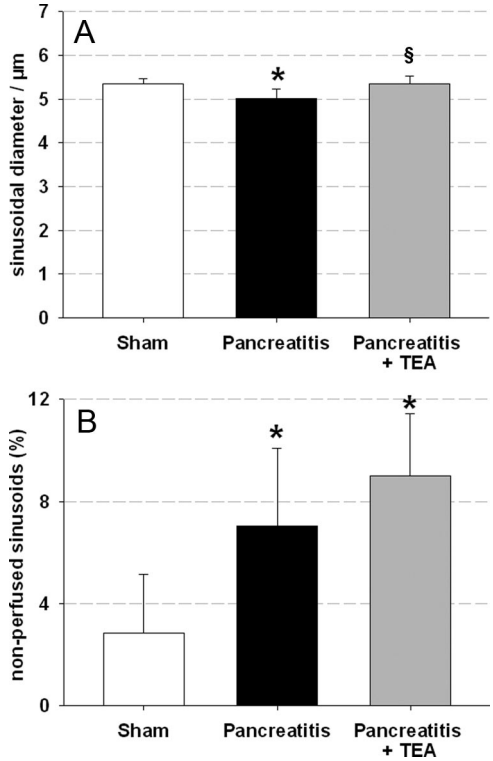

Fig. 1. Effects of thoracic epidural anesthesia on hepatic microcirculation in acute pancreatitis. Sinusoidal width (*A*) and percentage of nonperfused sinusoids (*B*) 15 h after induction of acute pancreatitis or sham procedure. In Pancreatitis, sinusoidal vasoconstriction occurred and sinusoidal perfusion was reduced (* $P < 0.05$ *vs.* Sham). Thoracic epidural anesthesia (TEA) prevented vasoconstriction (§ $P < 0.05$ *vs.* Pancreatitis) but could not influence loss of sinusoids. Data are presented as mean with the *error bar* denoting the 95% confidence interval; n = 7 in each group.

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

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

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.

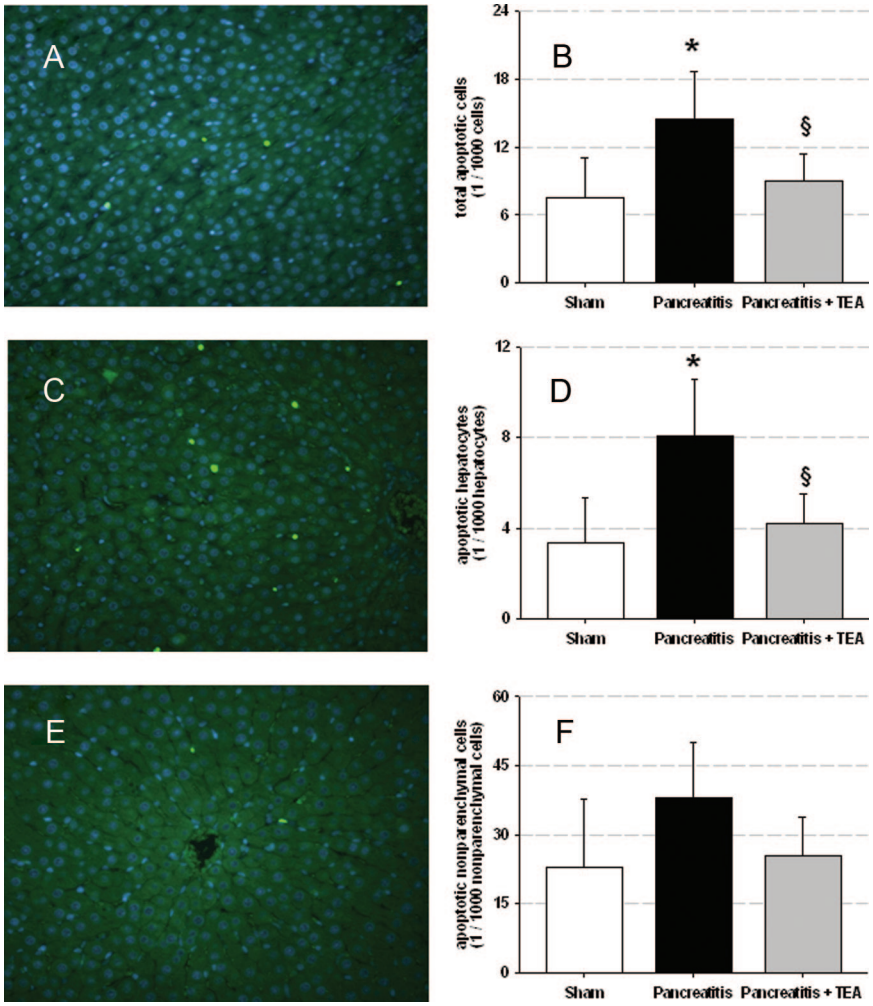

Fig. 2. Apoptosis. Single-strand DNA–positive cells (*green*) after 15 h in Sham (*A*), Pancreatitis (*C*), or Pancreatitis + Thoracic epidural anesthesia (TEA) (*E*). Nuclei are counterstained with 4′,6-diamidino-2-phenylindole (*blue*). Final magnification is 120-fold. The numbers of single-strand DNA–positive cells increased 15 h after induction of acute pancreatitis (* $P < 0.05$ *vs.* Sham). This increase was prevented in Pancreatitis + TEA (§ $P < 0.05$ *vs.* Pancreatitis) (*B*). These effects were also significant when hepatocytes were evaluated separately (*D*). In nonparenchymal cells (*F*), there was a trend toward a protective effect in Pancreatitis + TEA. Data are presented as mean with the *error bar* denoting the 95% confidence interval; n = 8 in Sham, and n = 7 in Pancreatitis and Pancreatitis + TEA.

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.

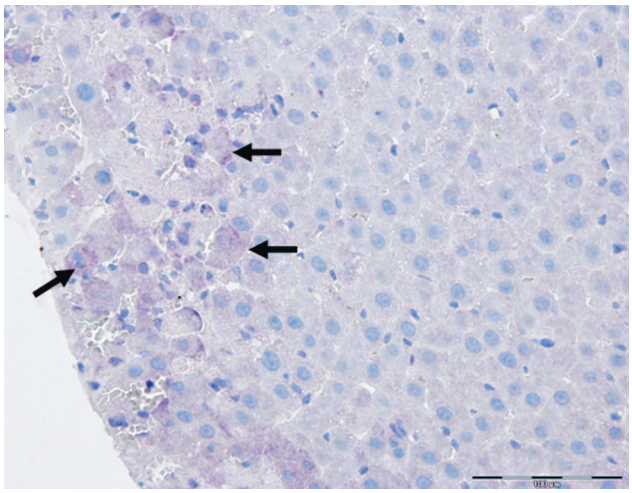

Fig. 3. Fas ligand expression. Intrahepatic Fas ligand expression (stained *red*) counterstained with hematoxylin–eosin 15 h after untreated pancreatitis. Final magnification is 360-fold.

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.[34] Hepatic oxygen delivery and tissue oxygenation remain constant during TEA in instrumented anesthetized pigs and dogs.[34,35]

Hepatic perfusion is subject to complex regulatory processes to which both sympathetic and parasympathetic activity contribute.[37,38] It is perfectly adjusted to maintain metabolic homeostasis,[39] an adapted systemic release of proteins, substrates, and mediators,[40] and to mobilize and retain intravascular blood volume.[41]

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,[42] 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.[43]

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 unrestricted animals.[44] 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.[5,14,45]

Microcirculation. Sinusoidal vasoconstriction and loss of sinusoidal perfusion occur early in systemic inflammation and contribute to organ injury.[46] Inflammation and impaired perfusion are associated with cellular hypoxia.[47] 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.[42] 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.[41,44,48] 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.[49]

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.[37,50] Under norepinephrine stimulation, isolated stellate cells contract and release proinflammatory cytokines in both α_1 and β receptor–dependent pathways.[51] Both stellate cells and epithelial cells can be activated by α-adrenergic stimulation.[52,53]

Because regulation of sinusoidal perfusion also occurs at both presinusoidal and postsinusoidal sites, the exact site of regulation cannot be identified in this study.[37]

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.[14] Inagaki *et al.*[54] 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.[46] 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.[46] 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.[55] In a recent study, accordingly, hepatic neutrophil recruitment started to decline after 8 h.[56] 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.[57,58] Hepatocyte apoptosis in acute pancreatitis is partly induced by FasL secretion by Kupffer cells.[59] However, Kupffer cells also undergo apoptosis during acute pancreatitis, thereby influencing systemic immune competence.[60] In models of hepatic ischemia–reperfusion and portal endotoxin challenge, Fas and FasL are also supposed to contribute to hepatic apoptosis.[61,62] Both sympathetic and parasympathetic activities affect the regulation of intrahepatic apoptosis and liver regeneration.[63]

However, experimental data are partially conflicting. On the one hand, Yu *et al.*[48] 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 β_2 adrenoceptors, norepinephrine application, and β-receptor stimulation reduced hepatocyte apoptosis in mice, rats, and hepatocyte cultures, respectively.[64,65] 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 thoracoabdominal sympathetic block.[23] 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.[66] These results suggest that TEA might protect the liver in the perioperative period and in critical illness.

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References

1. Wu CL, Hurley RW, Anderson GF, Herbert R, Rowlingson AJ, Fleisher LA: Effect of postoperative epidural analgesia on morbidity and mortality following surgery in medicare patients. Reg Anesth Pain Med 2004; 29:525–33

2. Tziavrangos E, Schug SA: Regional anaesthesia and perioperative outcome. Curr Opin Anaesthesiol 2006; 19:521–5

3. Rodgers A, Walker N, Schug S, McKee A, Kehlet H, van Zundert A, Sage D, Futter M, Saville G, Clark T, MacMahon S: Reduction of postoperative mortality and morbidity with epidural or spinal anaesthesia: Results from overview of randomised trials. BMJ 2000; 321:1493

4. Kehlet H: Manipulation of the metabolic response in clinical practice. World J Surg 2000; 24:690–5

5. Dhainaut JF, Marin N, Mignon A, Vinsonneau C: Hepatic response to sepsis: Interaction between coagulation and inflammatory processes. Crit Care Med 2001; 29:S42–7

6. Folch-Puy E: Importance of the liver in systemic complications associated with acute pancreatitis: The role of Kupffer cells. J Pathol 2007; 211:383–8

7. Fong YM, Marano MA, Moldawer LL, Wei H, Calvano SE, Kenney JS, Allison AC, Cerami A, Shires GT, Lowry SF: The acute splanchnic and peripheral tissue metabolic response to endotoxin in humans. J Clin Invest 1990; 85:1896–904

8. van de Poll MC, Derikx JP, Buurman WA, Peters WH, Roelofs HM, Wigmore SJ, Dejong CH: Liver manipulation causes hepatocyte injury and precedes systemic inflammation in patients undergoing liver resection. World J Surg 2007; 31:2033–8

9. Schemmer P, Schoonhoven R, Swenberg JA, Bunzendahl H, Thurman RG: Gentle *in situ* liver manipulation during organ harvest decreases survival after rat liver transplantation: Role of Kupffer cells. Transplantation 1998; 65:1015–20

10. Kotake Y, Takeda J, Matsumoto M, Tagawa M, Kikuchi H: Subclinical hepatic dysfunction in laparoscopic cholecystectomy and laparoscopic colectomy. Br J Anaesth 2001; 87:774–7

11. Kramer L, Jordan B, Druml W, Bauer P, Metnitz PG: Incidence and prognosis of early hepatic dysfunction in critically ill patients: A prospective multicenter study. Crit Care Med 2007; 35:1099–104

12. Harbrecht BG, Zenati MS, Doyle HR, McMichael J, Townsend RN, Clancy KD, Peitzman AB: Hepatic dysfunction increases length of stay and risk of death after injury. J Trauma 2002; 53:517–23

13. Derikx JP, Poeze M, van Bijnen AA, Buurman WA, Heineman E: Evidence for intestinal and liver epithelial cell injury in the early phase of sepsis. Shock 2007; 28:544–8

14. Gregory SH, Wing EJ: Neutrophil-Kupffer cell interaction: A critical component of host defenses to systemic bacterial infections. J Leukoc Biol 2002; 72:239–48

15. Xiao H, Siddiqui J, Remick DG: Mechanisms of mortality in early and late sepsis. Infect Immun 2006; 74:5227–35

16. Mentula P, Kylanpaa-Back ML, Kemppainen E, Takala A, Jansson SE, Kautiainen H, Puolakkainen P, Haapiainen R, Repo H: Decreased HLA (human leucocyte antigen)-DR expression on peripheral blood monocytes predicts the development of organ failure in patients with acute pancreatitis. Clin Sci (Lond) 2003; 105:409–17

17. Sanchez O, Viladrich M, Ramirez I, Soley M: Liver injury after an aggressive encounter in male mice. Am J Physiol Regul Integr Comp Physiol 2007; 293: R1908–16

18. Schemmer P, Enomoto N, Bradford BU, Bunzendahl H, Raleigh JA, Thurman RG: Autonomic nervous system and gut-derived endotoxin: Involvement in activation of Kupffer cells after *in situ* organ manipulation. World J Surg 2001; 25:399–406

19. Zhou M, Yang S, Koo DJ, Ornan DA, Chaudry IH, Wang P: The role of Kupffer cell alpha(2)-adrenoceptors in norepinephrine-induced TNF-alpha production. Biochim Biophys Acta 2001; 1537:49–57

20. Zhou M, Das P, Simms HH, Wang P: Gut-derived norepinephrine plays an important role in up-regulating IL-1beta and IL-10. Biochim Biophys Acta 2005; 1740:446–52

21. Yang S, Zhou M, Chaudry IH, Wang P: Norepinephrine-induced hepatocellular dysfunction in early sepsis is mediated by activation of alpha2-adrenoceptors. Am J Physiol Gastrointest Liver Physiol 2001; 281:G1014–21

22. Oben JA, Roskams T, Yang S, Lin H, Sinelli N, Li Z, Torbenson M, Huang J, Guarino P, Kafrouni M, Diehl AM: Sympathetic nervous system inhibition increases hepatic progenitors and reduces liver injury. Hepatology 2003; 38: 664–73

23. Freise H, Anthonsen S, Fischer LG, Van Aken HK, Sielenkamper AW: Continuous thoracic epidural anesthesia induces segmental sympathetic block in the awake rat. Anesth Analg 2005; 100:255–62

24. Brodner G, Van Aken H, Hertle L, Fobker M, Von Eckardstein A, Goeters C, Buerkle H, Harks A, Kehlet H: Multimodal perioperative management—combining thoracic epidural analgesia, forced mobilization, and oral nutrition—reduces hormonal and metabolic stress and improves convalescence after major urologic surgery. Anesth Analg 2001; 92:1594–600

25. Hogan QH, Stekiel TA, Stadnicka A, Bosnjak ZJ, Kampine JP: Region of epidural blockade determines sympathetic and mesenteric capacitance effects in rabbits. ANESTHESIOLOGY 1995; 83:604–10

26. Scott AM, Starling JR, Ruscher AE, DeLessio ST, Harms BA: Thoracic *versus* lumbar epidural anesthesia's effect on pain control and ileus resolution after restorative proctocolectomy. Surgery 1996; 120:688–95

27. Berendes E, Schmidt C, Van Aken H, Hartlage MG, Wirtz S, Reinecke H, Rothenburger M, Scheld HH, Schluter B, Brodner G, Walter M: Reversible cardiac sympathectomy by high thoracic epidural anesthesia improves regional left ventricular function in patients undergoing coronary artery bypass grafting: A randomized trial. Arch Surg 2003; 138:1283–90

28. Jorgensen H, Wetterslev J, Moiniche S, Dahl JB: Epidural local anaesthetics *versus* opioid-based analgesic regimens on postoperative gastrointestinal paralysis, PONV and pain after abdominal surgery. Cochrane Database Syst Rev 2000: CD001893

29. Kapral S, Gollmann G, Bachmann D, Prohaska B, Likar R, Jandrasits O, Weinstabl C, Lehofer F: The effects of thoracic epidural anesthesia on intraoperative visceral perfusion and metabolism. Anesth Analg 1999; 88:402–6

30. Sielenkamper AW, Eicker K, Van Aken H: Thoracic epidural anesthesia increases mucosal perfusion in ileum of rats. ANESTHESIOLOGY 2000; 93:844–51

31. Adolphs J, Schmidt DK, Korsukewitz I, Kamin B, Habazettl H, Schafer M, Welte M: Effects of thoracic epidural anaesthesia on intestinal microvascular perfusion in a rodent model of normotensive endotoxaemia. Intensive Care Med 2004; 30:2094–101

32. Freise H, Lauer S, Anthonsen S, Hlouschek V, Minin E, Fischer LG, Lerch MM, Van Aken HK, Sielenkamper AW: Thoracic epidural analgesia augments ileal mucosal capillary perfusion and improves survival in severe acute pancreatitis in rats. ANESTHESIOLOGY 2006; 105:354–9

33. Daudel F, Freise H, Westphal M, Stubbe HD, Lauer S, Bone HG, Van Aken H, Sielenkamper AW: Continuous thoracic epidural anesthesia improves gut mucosal microcirculation in rats with sepsis. Shock 2007; 28:610–4

34. Vagts DA, Iber T, Puccini M, Szabo B, Haberstroh J, Villinger F, Geiger K, Noldge-Schomburg GF: The effects of thoracic epidural anesthesia on hepatic perfusion and oxygenation in healthy pigs during general anesthesia and surgical stress. Anesth Analg 2003; 97:1824–32

35. Greitz T, Andreen M, Irestedt L: Haemodynamics and oxygen consumption in the dog during high epidural block with special reference to the splanchnic region. Acta Anaesthesiol Scand 1983; 27:211–7

36. Lerch MM, Weidenbach H, Gress TM, Adler G: Effect of kinin inhibition in experimental acute pancreatitis. Am J Physiol 1995; 269:G490–9

37. McCuskey RS: Morphological mechanisms for regulating blood flow through hepatic sinusoids. Liver 2000; 20:3–7

38. McCuskey RS: Anatomy of efferent hepatic nerves. Anat Rec A Discov Mol Cell Evol Biol 2004; 280:821–6

39. Jungermann K, Stumpel F: Role of hepatic, intrahepatic and hepatoenteral nerves in the regulation of carbohydrate metabolism and hemodynamics of the liver and intestine. Hepatogastroenterology 1999; 46 (suppl 2):1414–7

40. Lautt WW: The 1995 Ciba-Geigy Award Lecture: Intrinsic regulation of hepatic blood flow. Can J Physiol Pharmacol 1996; 74:223–33

41. Carneiro JJ, Donald DE: Change in liver blood flow and blood content in dogs during direct and reflex alteration of hepatic sympathetic nerve activity. Circ Res 1977; 40:150–8

42. Kurosawa M, Unno T, Aikawa Y, Yoneda M: Neural regulation of hepatic blood flow in rats: An *in vivo* study. Neurosci Lett 2002; 321:145–8

43. Ishikawa M, Yamataka A, Kawamoto S, Balderson GA, Lynch SV: Hemodynamic changes in blood flow through the denervated liver in pigs. J Invest Surg 1995; 8:95–100

44. Chida Y, Sudo N, Kubo C: Psychological stress impairs hepatic blood flow *via* central CRF receptors in mice. Life Sci 2005; 76:1707–12

45. Kmiec Z: Cooperation of liver cells in health and disease. Adv Anat Embryol Cell Biol 2001; 161:III–XIII, 1–151

46. Foitzik T, Eibl G, Hotz B, Hotz H, Kahrau S, Kasten C, Schneider P, Buhr HJ: Persistent multiple organ microcirculatory disorders in severe acute pancreatitis: Experimental findings and clinical implications. Dig Dis Sci 2002; 47:130–8

47. Fink MP: Bench-to-bedside review: Cytopathic hypoxia. Crit Care 2002; 6:491–9

48. Yu HJ, Lin BR, Lee HS, Shun CT, Yang CC, Lai TY, Chien CT, Hsu SM: Sympathetic vesicovascular reflex induced by acute urinary retention evokes proinflammatory and proapoptotic injury in rat liver. Am J Physiol Renal Physiol 2005; 288:F1005–14

49. Golling M, Jahnke C, Fonouni H, Ahmadi R, Urbaschek R, Breitkreutz R, Schemmer P, Kraus TW, Gebhard MM, Buchler MW, Mehrabi A: Distinct effects of surgical denervation on hepatic perfusion, bowel ischemia, and oxidative stress in brain dead and living donor porcine models. Liver Transpl 2007; 13:607–17

50. Pannen BH: New insights into the regulation of hepatic blood flow after ischemia and reperfusion. Anesth Analg 2002; 94:1448–57

51. Sancho-Bru P, Bataller R, Colmenero J, Gasull X, Moreno M, Arroyo V, Brenner DA, Gines P: Norepinephrine induces calcium spikes and proinflammatory actions in human hepatic stellate cells. Am J Physiol Gastrointest Liver Physiol 2006; 291:G877–84

52. Reinehr RM, Kubitz R, Peters-Regehr T, Bode JG, Haussinger D: Activation of rat hepatic stellate cells in culture is associated with increased sensitivity to endothelin 1. Hepatology 1998; 28:1566–77

53. Tighe D, Moss R, Bennett D: Cell surface adrenergic receptor stimulation modifies the endothelial response to SIRS (systemic inflammatory response syndrome). New Horiz 1996; 4:426–42

54. Inagaki H, Nakao A, Kurokawa T, Nonami T, Harada A, Takagi H: Neutrophil behavior in pancreas and liver and the role of nitric oxide in rat acute pancreatitis. Pancreas 1997; 15:304–9

55. Zhang P, Xie M, Spitzer JA: Hepatic neutrophil sequestration in early sepsis: Enhanced expression of adhesion molecules and phagocytic activity. Shock 1994; 2:133–40

56. Zhang H, Zhi L, Moochhala SM, Moore PK, Bhatia M: Endogenous hydrogen sulfide regulates leukocyte trafficking in cecal ligation and puncture-induced sepsis. J Leukoc Biol 2007; 82:894–905

57. Takeyama Y: Significance of apoptotic cell death in systemic complications with severe acute pancreatitis. J Gastroenterol 2005; 40:1–10

58. Wang YL, Hu R, Lugea A, Gukovsky I, Smoot D, Gukovskaya AS, Pandol SJ: Ethanol feeding alters death signaling in the pancreas. Pancreas 2006; 32:351–9

59. Gallagher SF, Yang J, Baksh K, Haines K, Carpenter H, Epling-Burnette PK, Peng Y, Norman J, Murr MM: Acute pancreatitis induces FasL gene expression and apoptosis in the liver. J Surg Res 2004; 122:201–9

60. Peng Y, Gallagher SF, Haines K, Baksh K, Murr MM: Nuclear factor-κB mediates Kupffer cell apoptosis through transcriptional activation of Fas/FasL. J Surg Res 2006; 130:58–65

61. Choda Y, Morimoto Y, Miyaso H, Shinoura S, Saito S, Yagi T, Iwagaki H, Tanaka N: Failure of the gut barrier system enhances liver injury in rats: Protection of hepatocytes by gut-derived hepatocyte growth factor. Eur J Gastroenterol Hepatol 2004; 16:1017–25

62. Nakajima H, Mizuta N, Fujiwara I, Sakaguchi K, Ogata H, Magae J, Yagita H, Koji T: Blockade of the Fas/Fas ligand interaction suppresses hepatocyte apoptosis in ischemia-reperfusion rat liver. Apoptosis 2008; 13:1013–21

63. Kiba T: The role of the autonomic nervous system in liver regeneration and apoptosis: Recent developments. Digestion 2002; 66:79–88

64. Andre C, Couton D, Gaston J, Erraji L, Renia L, Varlet P, Briand P, Guillet JG: Beta2-adrenergic receptor-selective agonist clenbuterol prevents Fas-induced liver apoptosis and death in mice. Am J Physiol 1999; 276:G647–54

65. Hamasaki K, Nakashima M, Naito S, Akiyama Y, Ohtsuru A, Hamanaka Y, Hsu CT, Ito M, Sekine I: The sympathetic nervous system promotes carbon tetrachloride-induced liver cirrhosis in rats by suppressing apoptosis and enhancing the growth kinetics of regenerating hepatocytes. J Gastroenterol 2001; 36:111–20

66. Ueno T, Bioulac-Sage P, Balabaud C, Rosenbaum J: Innervation of the sinusoidal wall: Regulation of the sinusoidal diameter. Anat Rec A Discov Mol Cell Evol Biol 2004; 280:868–73