Thoracic Epidural Analgesia Augments Ileal Mucosal Capillary Perfusion and Improves Survival in Severe Acute Pancreatitis in Rats

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Background: Acute pancreatitis has been linked to intestinal barrier dysfunction and systemic inflammatory response with high mortality. Thoracic epidural analgesia improves intestinal perfusion. The authors hypothesized that thoracic epidural analgesia influences microcirculation injury, inflammatory response, and outcome of acute pancreatitis in rats.

Methods: Control groups underwent a sham procedure or untreated pancreatitis induced by intraductal taurine injection. In the treatment groups, epidural analgesia was commenced immediately after a 7-h delay. Fifteen hours after injection, ileal mucosal perfusion was assessed by intravital microscopy. Thereby, the intercapillary area between all perfused capillaries and between continuously perfused capillaries was determined by differentially quantifying total and continuous capillary mucosal perfusion. Villus blood flow and serum levels of amylase, lactate, and interleukin 6 were determined, and pancreatic injury was scored histologically. Seven-day survival was recorded in an additional 30 rats undergoing untreated pancreatitis or pancreatitis with epidural analgesia.

Results: In untreated pancreatitis, decreased total capillary perfusion increased the total intercapillary area by 24%. Furthermore, loss of continuous perfusion increased continuous intercapillary area to 228%. After immediate and delayed epidural analgesia, continuous perfusion was restored (P < 0.05). Blood flow decreased 50% in untreated pancreatitis but was preserved by epidural analgesia (P < 0.05). Biochemical and histologic signs of pancreatitis were not affected by epidural analgesia. Lactate and interleukin-6 levels increased in untreated pancreatitis, which was prevented in the treatment groups (P < 0.05). Epidural analgesia increased 7-day survival from 33% to 73% (P < 0.05).

Conclusion: Thoracic epidural analgesia attenuated systemic inflammatory response and improved survival in severe acute pancreatitis. These effects might be explained by improved mucosal perfusion.

THE mortality of severe acute pancreatitis (AP) is still high, reaching up to 25%. Morbidity and mortality are determined by the development of a systemic inflammatory response, infection of pancreatic necroses, and multiple organ failure. The intestinal tract might contribute to systemic complications of the disease. Disturbed intestinal permeability is observed in both animal models and humans. Endotoxemia and bacterial translocation from the intestinal tract with subsequent infection of pancreatic necroses are considered to be of major importance in the disease. Mucosal hypoperfusion was documented in small patient series with mild and severe AP and has been shown to predict organ failure and outcome. Experimental studies revealed intestinal microcirculatory injury both in edematous and in necrotizing pancreatitis. Approaches to maintain gastrointestinal barrier function in AP included enteral feeding and glutamine supplementation.

Thoracic epidural analgesia (TEA) reduces postoperative mortality and morbidity compared with general anesthesia. TEA also modifies the metabolic response to surgical stress. Furthermore, TEA improves intestinal recovery after major surgery. In experimental settings, TEA has been shown to improve capillary mucosal perfusion in the presence of systemic hypotension and to attenuate hypoxemic intestinal damage. A segmental sympathetic block with blood flow redistribution toward the splanchnic vascular bed is supposed to mediate some of these beneficial effects of TEA.

In severe AP, effective pain therapy can be provided by TEA. Beyond this, however, TEA might ameliorate intestinal perfusion injury and affect the systemic response to pancreatic injury. In the presented study, continuous TEA was therefore applied to reduce intestinal microvascular injury and possibly improve outcome in a model of severe AP in rats.

Materials and Methods

The study was approved by the animal ethics committee of the district government of Muenster, Muenster, Germany. Animals received standard chow and were kept on a 12-h light–dark cycle. Male rats (275–300 g; Harlan-Winkelmann, Borchen, Germany) were anesthetized with isoflurane in 50% oxygen. Central venous and arterial lines (0.96 mm OD; Liquidscans, Ueberlingen, Germany) were introduced. Epidural catheters (0.61 mm OD; Liquidscans) were inserted at L3–L4 and advanced to Th6. All catheters were inserted at L3–L4 and advanced to Th6.
were exteriorized at the neck and protected by a swivel device. The pancreas was exposed, and the proximal bile duct was temporarily clamped. AP was induced by retrograde intraductal injection of 2 ml/kg taurocholate, 5% (Sigma-Aldrich Chemie GmbH, Munich, Germany). After abdominal closure, the rats were then allowed to wake up. Intravenous saline, 2 ml/h, was infused throughout the experiment to prevent hypovolemia.

**Microcirculation Protocol**

Twenty-eight animals were randomly assigned to four groups: sham procedure, 15 µl/h NaCl 0.9% epidurally (Sham); AP, 15 µl/h NaCl 0.9% epidurally (PANC); AP, 15 µl/h bupivacaine 0.5% epidurally (EPI); and AP, 15 µl/h bupivacaine 0.5% epidurally after a 7-h delay (delayed EPI). The investigators were not aware of the group assignment.

Fifteen hours after injury, mean arterial blood pressure was recorded using a standard transducer (PMSSET 1DT; Becton Dickinson, Heidelberg, Germany) and a monitor (Siemens Sirecust 404; Siemens, Munich, Germany). Heart rate was recorded using the arterial pressure curve.

Muscular tone and function was quantified using an established motor score that was derived from Bromage score and adapted to rats. Categories of this score are as follows: 0 = no motor deficit, normal function; 1 = mild reduction in muscular tone of the hind limb, able to stand on the hind limbs but reduced power and control when moving; 2 = moderately reduced power, difficulties with standing on hind limb; 3 = complete loss of power, flat body posture. Briefly, the animals were then reanesthetized, tracheotomized, and ventilated (Harvard Apparatus, Holliston, MA). Intravital microscopy of the ileal mucosa was performed as described previously. The animals were then reanesthetized, tracheotomized, and ventilated (Harvard Apparatus, Holliston, MA). Intravital microscopy of the ileal mucosa was performed as described previously. Briefly, a segment of the distal ileum was cut at the antimesenteric side, and the luminal face was placed on the intravital microscope (Eclipse 300; Nikon, Düsseldorf, Germany). Intravenous fluorescein isothiocyanate-albumin, 30 mg/kg (Sigma-Aldrich Chemie GmbH), was injected for enhancement of erythrocyte–tissue contrast. The temperature of the preparation was kept normothermic with warm saline solution. After a stabilization period of 20 min, 6-10 villi were recorded for 1 min onto videotape using a 740-fold magnification.

**Video Analysis**

Quality of mucosal microcirculation was evaluated by total and continuous capillary perfusion as described previously. Briefly, all perfused capillaries were manually traced on a transparent sheet. Perfusion was defined as passage of at least one erythrocyte during the recording period. The sheets were scanned to a computer, and the intercapillary area (ICA) enclosed by all perfused capillaries (ICA_total) was determined (AnalySIS; Soft Imaging System, Muenster, Germany). Capillaries with intermittent flow were then identified, and ICA was assessed again including only the continuously perfused capillaries (ICA_cont). The ICA is inversely proportional to the density of perfused capillaries.

Quantitative evaluation of mucosal blood flow was performed by calculating flow in the terminal mucosal arterioles. Terminal arterioles were identified, and the arteriolar diameter was estimated by the average of five measurements at different segments of the vessel. Arteriolar erythrocyte velocity was assessed using frame-by-frame analysis (Δt 40 ms) of the traveled distance. Arteriolar blood flow (Q) was then calculated by the equation $Q = \pi \times (\text{arteriolar diameter}^2/4) \times \text{arteriolar erythrocyte velocity}$. 

**Blood Sampling and Analysis**

Blood was collected by aortic puncture after intravital microscopy ($n = 7$ per group). Serum lactate levels were determined by blood gas analysis (Radiometer ABL, Copenhagen, Denmark). Serum amylase activity was measured photometrically with CobasBio (Roche, Mannheim, Germany). Interleukin 6 (IL-6) was determined by enzyme-linked immunosorbent assay (R&D, Minneapolis, MN).

**Histology**

Pancreatic tissue was formalin fixed and paraffin embedded ($n = 7$ per group). One thin section per animal was stained by hematoxylin–eosin and evaluated by a blinded pathologist. Pancreatic injury was assessed by scoring hemorrhage, interlobular and intralobular infiltration, and necrosis. Each item was graded from 0 (absence) to 3 (severe) points for each slide, and points were added.

**Outcome Protocol**

An additional 30 rats were randomly assigned to PANC ($n = 15$) and EPI ($n = 15$). Epidural analgesia or epidural saline infusion respectively was commenced immediately after induction of pancreatitis and continued until the death of the animal or the end of the 7-day observation period. The rats had access to food. Saline solution, 2 ml/h, was infused throughout the observational period. Survival was observed for 7 days.

**Statistical Analysis**

SigmaStat 3.0 (Systat Software GmbH, Erkraft, Germany) was used for statistical analysis. Data were tested for normality and equal variance. Pancreatitis-induced effects were evaluated by $t$ test between Sham and PANC. Treatment effects of the two different treatment protocols were compared with PANC by analysis of variance with post hoc Student-Newman-Keuls test or analysis of variance on ranks followed by post hoc Dunn
**Results**

All animals in the microcirculation protocol survived the observational period of 15 h. Heart rate (389 ± 17 vs. 378 ± 28 beats/min) and mean arterial pressure (118 ± 4 vs. 112 ± 4 mmHg) remained stable in PANC compared with Sham. Neither in EPI (heart rate, 340 ± 11 beats/min; mean arterial pressure, 128 ± 11 mmHg) nor in delayed EPI (heart rate, 354 ± 19 beats/min; mean arterial pressure, 111 ± 12 mmHg) did TEA induce changes of hemodynamic parameters in the presence of pancreatitis. In EPI and delayed EPI, only mild motor deficits occurred (motor score 0 [0, 1] in both groups).

**Pancreatic Injury**

In Sham, no signs of pancreatic injury were recorded (0 [0, 0]). In the PANC group, histologic examination showed signs of necrosis, intraparenchymal hemorrhage, and inflammation with a median score of 4 (3, 10), (P < 0.05 vs. Sham) (fig. 1A). In the treatment groups, signs of pancreatitis were reduced to 0 (0, 2) in EPI and 0 (0, 4) in delayed EPI (fig. 1B). Statistical analysis by analysis of variance on ranks revealed a trend toward reduced histologic pancreatic injury (P = 0.06). In PANC, serum amylase increased from 872 ± 67 to 4247 ± 545 U/l (P < 0.05). TEA did not influence amylase plasma levels in EPI (3,703 ± 661 U/l) or delayed EPI (4,546 ± 510 U/l).

**Microcirculation**

In the PANC group, an increase in ICA_{total} (936 ± 69 vs. 757 ± 42 m^2; P < 0.05) was recorded, indicating a decrease in capillary perfusion (fig. 2A). This effect was not influenced in the EPI or delayed EPI groups compared with PANC. After the induction of AP, continuous capillary perfusion decreased, as indicated by increased ICA_{cont} (2,996 ± 415 m^2 in PANC vs. 1,310 ± 226 m^2 in Sham; P < 0.05). In the EPI group, continuous perfusion was normalized with ICA_{cont} reduced to 1,641 ± 397 m^2 (P < 0.05 vs. PANC). In the delayed EPI group, intermittent capillary perfusion was also attenuated to 1,355 ± 151 m^2 (P < 0.05 vs. PANC) (fig. 2B).

In the PANC group, a decrease in arteriolar blood flow (10,382 ± 1,417 vs. 21,087 ± 2,819 m^2/s; P < 0.05 vs. Sham) was demonstrated. Epidural infusion of bupivacaine restored blood flow (18,465 ± 2,805 m^2/s; P < 0.05 vs. PANC) (fig. 3). In the delayed EPI group, blood flow was not significantly augmented.

**Serum Lactate**

In the PANC group, serum lactate levels increased from 3.4 ± 0.5 to 5.4 ± 1.2 mm (P < 0.05 vs. Sham).

**Fig. 1.** Hematoxylin–eosin staining of paraffin-embedded pancreas 15 h after induction of acute pancreatitis. Final magnification 200-fold. A shows extensive necrosis and hemorrhage in untreated pancreatitis (PANC). In B, signs of pancreatic tissue damage are reduced but still present. EPI = epidural analgesia.
Epidural infusion of bupivacaine reduced lactate levels to $3.48 \pm 0.85$ mmol/l in the EPI group ($P < 0.05$ vs. PANC) and $2.3 \pm 0.6$ mmol/l in the delayed EPI group ($P < 0.05$ vs. PANC). There was no significant difference in the lactate levels between EPI and delayed EPI.

**Serum Interleukin 6**

Serum levels of the proinflammatory mediator IL-6 were increased in the PANC group ($142 \pm 10$ ng/ml vs. $82 \pm 9$ ng/ml; $P < 0.05$ vs. Sham). In the EPI group, IL-6 level was $119 \pm 13$ ng/ml. In the delayed EPI group, IL-6 level decreased to $99 \pm 9$ ng/ml ($P < 0.05$ vs. PANC). There was no significant difference in the IL-6 levels between EPI and delayed EPI.

**Survival**

In the outcome protocol, the 7-day survival rate in the PANC group was 33%. In the EPI group, 73% of the animals survived ($P < 0.05$ vs. PANC) (fig. 4). Most animals died between 36 and 48 h after induction of AP. The cause of death was not determined. In both groups, only one animal died at a later time.

**Discussion**

In this study, AP was associated with disturbance of the small bowel mucosal microcirculation, which was characterized by intermittent capillary perfusion and a substantial decrease in arteriolar blood flow. TEA improved arteriolar blood flow and capillary perfusion in the gut mucosa. In this respect, TEA was also effective when applied as a delayed treatment. The protective effects of TEA were associated with reduced serum lactate levels. Serum IL-6 level was decreased in delayed treatment. In addition, mortality from AP was reduced by 66% when TEA was applied.

Severe hypovolemia is a common finding in patients presenting with AP. Previously, others have demonstrated severe hypovolemia in a model of AP as indicated by oliguria, increased hematocrit, and acidosis. Because such hypovolemia would severely affect intestinal microcirculation, 2 ml/h saline was infused to prevent confusion of AP-induced injury with the hypovolemic shock. This regimen has been shown to preserve normovolemia in rats subjected to necrotizing AP. In the presented study, hemodynamic parameters did not change in untreated pancreatitis, indicating a sufficient volume replacement. Furthermore, TEA induced no significant changes in mean arterial pressure and heart rate when applied in critically ill rats. During continuous experimental TEA without initial bolus dose, in healthy animals, stable hemodynamic parameters have been demonstrated previously. This technique induces a gradual onset of sympathetic block and allows hemodynamic compensation by vasoconstriction outside the blocked segments.

Gastrointestinal injury is a key factor in the development of systemic inflammatory and infectious complications in AP. Mucosal barrier function is impaired in AP with increased permeability to both micromolecules and macromolecules. Gut-derived microorganisms are the most frequent pathogens in infected pancreatic necroses, and intestinal colonization precedes pancreatic infection by the same microorganisms. Preventive strategies to maintain mucosal integrity and prevent bacterial translocation such as enteral nutrition, supplementation of glutamine, or selective digestive decontamination are successfully applied in the treatment of AP.

Mucosal hypoperfusion may be crucial in the pathophysiology of gut barrier dysfunction. In clinical studies, impaired tissue oxygenation as measured by decreased intramucosal pH has been demonstrated both in mild and in severe AP. In rat models of edematous and necrotizing AP, microvascular injury was described in colon mucosa, liver, and lung. In this study, impaired capillary perfusion and reduced mucosal blood flow was demonstrated in a sufficiently resuscitated model of AP. Similar alterations in mucosal perfusion have been demonstrated earlier in polymicrobial sepsis, which is also known to be associated with mucosal ischemia and gut barrier failure. The AP-induced changes in mucosal microcirculation may lead to local hypoxia at the villus tip, because the reduced inflow was distributed heterogeneously within the capillary network.

Continuous TEA prevented the AP-induced intermittent capillary perfusion. Improved capillary perfusion was associated with an increase in gut mucosal blood flow as measured by mucosal arteriolar blood flow. There are clinical and experimental data suggesting such a protective effect of TEA on the gut. In healthy rats, TEA improved capillary perfusion despite mild systemic hypotension. In rabbits subjected to progressive hypoxemia, TEA improved mucosal oxygenation and positively influenced the portal endotoxin concentration as a marker of barrier dysfunction and bacterial translocation. TEA also prevented an intraoperative decrease in gastric mucosal pH under clinical conditions.
function and motility are improved when TEA is applied in the perioperative period.\textsuperscript{16,17}

The beneficial effects of TEA may be attributable to segmental sympathetic block with subsequent blood flow redistribution from the segments with maintained or increased sympathetic activity toward the intestinal tract. Others reported that TEA increased sympathetic activity caudal to the blocked segments in the kidney in cats. Baroreceptor-dependent sympathetic reflexes contributed to hemodynamic stability during segmental sympathetic block in this study.\textsuperscript{32} In rabbits, decreased sympathetic activity was demonstrated in mesenteric vessels during TEA while lumbar epidural analgesia increased intestinal sympathetic activity.\textsuperscript{33} In those studies that did not show beneficial effects of TEA on visceral perfusion, the measurements were performed outside the blocked segments.\textsuperscript{34,35} In our model of TEA, we recently demonstrated a segmental increase in skin temperature in the thoracoabdominal region with decreased temperature in the more caudal regions, suggesting a segmental sympathetic block with increased sympathetic activity outside of the blocked region.\textsuperscript{22} In the presented study, the block included both the pancreas and the distal ileum.

Little is known about the effect of TEA in critical illness, because most data regarding the effect of TEA on intestinal perfusion are derived from studies in healthy animals and in patients undergoing elective surgery. In a clinical study in critically ill patients, gastrointestinal motility improved during TEA.\textsuperscript{30} In contrast to this, a redistribution of ileal blood flow away from the mucosa to the muscularis during endotoxemia was reported from a rat study, therefore questioning the use of TEA in critical illness.\textsuperscript{37} In the latter study, a 30-\textmu l bolus followed by 100 \textmu l/h lidocaine was applied. This high dose may have induced a nonsegmental block and may have masked beneficial effects of TEA on the mucosal microcirculation.

The presented study is the first to show beneficial effects of TEA on gut mucosal perfusion in a model of critical illness. Protection of capillary perfusion was also demonstrated in delayed treatment, which mimics the clinical situation of patients presenting with fully developed disease.

In AP, IL-6 is released in high concentration at the site of the injury.\textsuperscript{38} The systemic release of IL-6 predicts lung injury and adverse outcome of the disease.\textsuperscript{39,40} IL-6 impairs gut mucosal integrity by decreasing the expression of tight junction proteins and decreases bowel motility.\textsuperscript{41,42} Increased lactate levels are a frequent finding in AP and may in part represent impaired tissue oxygenation.\textsuperscript{43,44} Persistent increase of serum lactate is associated with poor outcome in critical illness.\textsuperscript{45,46}

In this study, an increase in serum IL-6 and in serum lactate levels was recorded in PANC. TEA partly modified the inflammatory response as represented by reduction of IL-6-levels in delayed EPI. Both in immediate and in delayed treatment, TEA reduced elevated lactate levels, possibly representing improved overall tissue oxygenation.

It is an important finding of this study that TEA not only improved mucosal capillary perfusion and modified systemic response to AP but also was associated with a 66\% decrease of mortality.

Although this study provides possible explanations for the observed beneficial effects of TEA, such as a reduction in systemic inflammation, augmented microvascular blood flow, and improved tissue oxygenation, we cannot explain the exact reason for these effects of TEA.

One may speculate that an initially reduced inflammatory response might result in improved microvascular perfusion. In this respect, systemic absorption of epidurally administered bupivacaine could play a role, because a recent study reported dose-dependent beneficial effects of systemically applied bupivacaine on inflammatory response, renal dysfunction, and survival in murine sepsis.\textsuperscript{37}

Because enteral nutrition has been demonstrated to improve morbidity and mortality in AP,\textsuperscript{13} the beneficial effect of TEA on mortality from AP could also be related to increased food intake. In this study, food intake was not recorded in the outcome protocol. However, the observed beneficial effects on mucosal perfusion and systemic inflammatory response were obviously independent of enteral food intake, because in the microcirculation protocol, the animals had no access to food during the observational period.

The use of TEA in critical ill patients has gained increasing interest.\textsuperscript{20,36,48} This study proved that TEA reduced intestinal injury, modified systemic inflammatory response, and reduced mortality in a model of severe AP. Even when TEA was initiated after a delay when AP was fully established, gut mucosal perfusion injury was ameliorated. Pancreatic injury was not affected. These results suggest that TEA might be an effective therapeutic strategy to reduce systemic complications of AP.

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