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Inflammatory profiling of early experimental necrotizing pancreatitis

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ABSTRACT

Aims: Inflammatory mediators play a pivotal role in severe necrotizing pancreatitis (SNP). Therapeutic approaches aim at the early inflammatory liberation of cytokines to avoid systemic complications. The present study evaluates the kinetics of inflammatory mediator release in SNP.
Main methods: Experimental SNP was induced in male Wistar rats using the GDOC model. The animals were allocated into seven groups (n = 6/group). In group 1, sample harvesting was performed after sham operation while in groups 2–7 this was performed 1 h, 2 h, 4 h, 6 h, 9 h, and 12 h after initiation of SNP, respectively. Inflammatory mediator release, morphologic injury, and tissue MPO concentrations were evaluated between 1 and 12 h after induction.
Key findings: Pancreatic injury showed a continuous increase over the observation period ($p < 0.05$, respectively). MPO levels in the pancreas and lungs increased until 12 h after induction ($p < 0.05$, respectively). Anti-inflammatory IL-10 showed an early peak and the pro-inflammatory mediators TNFα and IL-1β peaked after 6 and 9 h, respectively ($p < 0.05$, respectively). HMGB1 levels constantly increased over time ($p < 0.05$, respectively).
Significance: The present study shows the release of relevant pro- and anti-inflammatory mediators in SNP for the first time in one single experimental setup. Inflammatory mediators peak within the first few hours after SNP induction. Consequently, the effect of therapeutic approaches on early changes in cytokine release should be evaluated later than 2 h after initiation.

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Introduction

Severe necrotizing pancreatitis (SNP) remains a disease without a specific causal therapy and is associated with significant mortality rates of up to 30% [1,2]. Inflammatory mediators trigger its development from a local to a systemic disease including systemic inflammatory response syndrome (SIRS) and distant organ dysfunction with renal, cardiovascular, and pulmonary insufficiency associated with relevant morbidity and mortality rates [1,3,4]. This progression from a local to a systemic disease is initiated by the release of pro-inflammatory mediators into the blood [5]. Various promising experimental approaches interfering with these mediators have failed to show beneficial effects in the clinical setting [6–23]. One major problem is that applied prophylactic agents ameliorate pancreatic damage but are ineffective when administration is delayed until after establishment of SNP [24]. However, no prophylactic application is possible in the clinical setting, except for post-ERCP and post-transplant pancreatitis. The therapeutic window acting against liberation of inflammatory mediators in human acute pancreatitis has been described as lasting for three days after the onset of symptoms. The experimental setting allows the investigation of these mechanisms in a shortened timeframe [5].

The aim of the present study was to evaluate the longitudinal time course of the early release of known relevant inflammatory mediators in experimental SNP in correlation with morphological changes including pulmonary injury in one single experimental setup. There is no data available in the current literature, which describes these findings in the used experimental model. These observations would then be correlated with the presumed three-day opportunity window of therapeutical antagonism of the proinflammatory mediator in the clinical setting to define the ideal therapeutic window for future drug investigation in this experimental setting.

Materials and methods

The present trial was approved by the Regierungspräsidium Karlsruhe.

Anesthesia and catheter placement are described in detail elsewhere [25]. In brief, after general anesthesia a catheter was placed in the left carotid artery and in the right internal jugular vein. The arterial catheter was used for hemodynamic monitoring, whereas the venous catheter was used for drug application.

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Severe necrotizing pancreatitis (SNP) was induced with the established GDOC model described in detail elsewhere [25,26].

Experimental design

The experiments were conducted on male Wistar rats. There were seven groups with six animals per group. In group 1, systemic blood samples and organ specimens were harvested directly after sham operation and no induction of SNP as a control group. In groups two to seven, blood and organ samples were harvested 1, 2, 4, 6, 9, and 12 h after initiation of SNP, respectively.

Morphologic organ damage

Morphologic damage was assessed on hematoxylin and eosin (H&E) stained histological slices of the pancreatic head. The staining process is described in detail elsewhere [25]. Evaluation of the fixed slices was performed blinded for the experimental groups. The scoring system regarding edema, inflammation, and necrosis was conducted as described by Schmidt et al. [26].

Pulmonary and pancreatic myeloperoxidase assay (MPO)

Pulmonary and pancreatic tail specimens were shock-frozen in liquid nitrogen and stored at −80 °C. Thereafter, the tissue samples were further processed after homogenization for evaluation of tissue MPO levels. The technique for homogenization and MPO analysis has been described elsewhere [27]. Via spectroscopy the MPO amount was indicated in U/mg protein.

Inflammatory mediators

HMGB1 in serum samples

HMGB1 levels were assessed with an immunoassay-HMGB1 according to the manufacturer's recommendations (IBL International GmbH, Hamburg, Germany) and the process is described elsewhere in detail [25]. HMGB1 levels were presented in ng/mL.

Serum samples for cytokines IL-1β, IL-6, IL-10 and TNF-α were analyzed by means of Bio-Plex Pro™ rat assays (cytokine, chemokine and growth factor, Bio-Plex; Bio-Rad; Hercules, CA) in accordance with the manufacturer's recommendations.

Cytokine concentrations (pg/mL) were analyzed automatically with Bio-Plex manager ™6.0 software with 5PL curve fitting.

Data analysis and statistics

Data were presented as means ± standard error of the mean (SEM). Data were analyzed with Microsoft Windows Excel for Mac 2011 Software (Version 14.3.5) and StatPlus:mac2009. Differences between groups were compared with one-way ANOVA followed by a post hoc t-test for analysis of significance levels between the groups. Statistical significance was defined at the 5% level ($P < 0.05$).

Results

Pancreatic morphologic evaluation

In healthy control animals, no edema, inflammation, or necrosis was observed, and morphological changes were consecutively classified by the score "0". Relevant edema formation emerged 1 h after initiation of SNP ($p = 0.03$) compared with healthy controls and increased further after 12 h.

Pancreatic inflammation showed a significant increase in leukocyte infiltration starting 2 h after induction of SNP ($p = 0.022$) with a continuous increase until 12 h (6 h: $p < 0.0005$ vs. control; $p < 0.05$ vs. 4 h; 12 h: $p = 0.031$ vs. 6 h).

There was no development of necrosis evident at 2 h and 4 h compared with healthy controls, whereas there was significant formation of necrosis 6 h after initiation of SNP ($p < 0.0005$). After 12 h, necrosis formation showed a significant further increase compared with 6 h ($p = 0.0006$) (Fig. 1a, b).

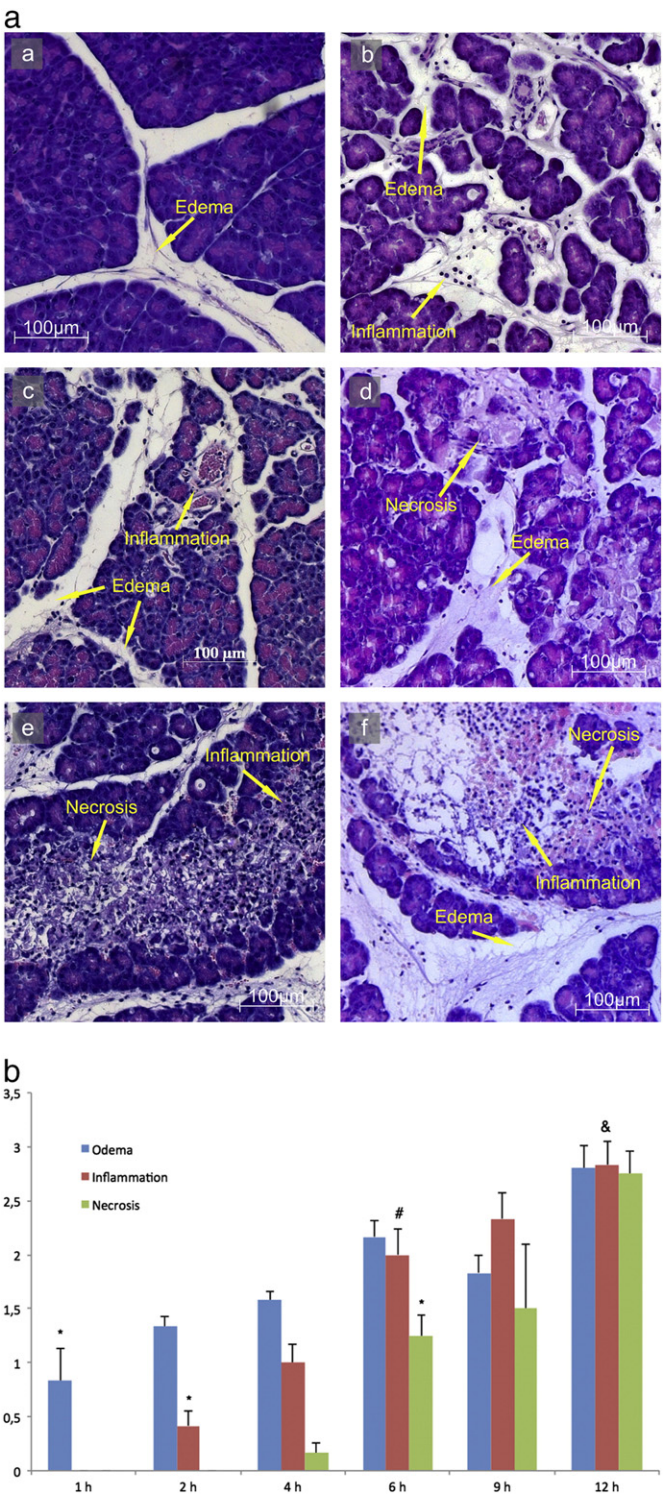

Fig. 1. a/b. Morphologic pancreatic injury. Pancreatic edema was present after 1 h with a further increase up to 12 h. Inflammation showed a significant increase 2 h after initiation of SNP with a further increase up to 12 h. Pancreatic necrosis was visible after 6 h with a further increase up to 12 h compared with healthy controls. Pancreatic necrosis developed 6 h after initiation of disease. Samples were stained using hematoxylin and eosin. Data expressed as mean ± SEM. *$p < 0.05$ vs. controls, #$p < 0.001$ vs. control and $p < 0.05$ vs. 4 h; &$p < 0.05$ vs. 6 h; §$p < 0.001$ 12 h vs. 6 h.

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