Acute lung injury in acute pancreatitis — Awaiting the big leap

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Summary
Acute lung injury is a severe complication to acute pancreatitis and a significant health problem associated with a considerable mortality. Underlying mechanisms are complex and poorly understood, although recent insights have identified several inflammatory profiles and cellular components involved to varying degrees during different phases of pancreatitis exacerbation and acute lung injury. This review aims to highlight the current understanding of the inflammatory and cellular components involved in and responsible for the associations of acute pancreatitis and acute lung injury, with the hope of thereby providing an increased understanding of the underlying mechanisms. In addition, novel experimental models of modulating the pancreatitis-associated acute lung injury are presented, interventions that may be of potential future clinical value.

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Abbreviations: AP, Acute pancreatitis; SAP, Severe acute pancreatitis; ALI, Acute lung injury; ARDS, Acute respiratory distress syndrome; MPO, Myeloperoxidase; TNF-α, Tumor necrosis factor-alpha; SIRS, Systemic inflammatory response syndrome; ICAM-1, Intercellular adhesion molecule-1; MCP-1, Monocyte chemoattractant protein-1; HSP72, Heat shock protein 72; RNI, Reactive nitrogen intermediates; ROI, Reactive oxygen intermediates.

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Background

Acute pancreatitis (AP) is a condition with an annual incidence of approximately 300 cases/million inhabitants in the Western world. The majority of cases are classified as mild according to the Atlanta Classification, while about 15% have severe acute pancreatitis with an associated mortality of around 10%.

Hypovolemia (<100 mmHg) at admission is among predictive factors affecting the course of disease, which correlates with both morbidity and increased mortality in patients with predicted severe acute pancreatitis. In addition, there appears to be a correlation between hypovolemia and the magnitude of the systemic inflammatory response syndrome (SIRS), in which e.g. increased endothelial barrier permeability and profound release of pro-inflammatory chemokines and cytokines may cause organ dysfunction. It has thereby been acknowledged that early and carefully monitored fluid resuscitation influence the course of disease, probably by minimizing ischemia and reperfusion injury, and the concomitant pro-inflammatory response.

Acute respiratory failure comprises both acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), both with typical radiological findings as well as physiological changes. The arterial oxygen pressure and inspired oxygen concentration ratio \((P_{aO_2}/F_{IO_2})\) is utilized to distinguish the two conditions, where ALI is defined as \((P_{aO_2}/F_{IO_2}) \leq 300 \text{ mmHg and ARDS} \leq 200 \text{ mmHg}.\) Overall, ALI and ARDS are significant health problems accounting for mortality rates between 30 and 40%. Approximately 190,000 ALI cases are reported annually in the US, responsible for up to 74,500 deaths per year. Common causes include sepsis, pneumonia, trauma, and acute pancreatitis. In severe acute pancreatitis, acute lung failure is the main contributing factor to early deaths, i.e. within the first week after admission, by developing single or multiple organ dysfunction. It has also been reported that ALI is the consequence of the systemic inflammatory response, which also regulates the magnitude of the pulmonary injury, involving granulocytes, macrophages, cytokines/chemokines and intracellular signaling pathways, which are all the topics which will be highlighted in this review.

ALI and ARDS in acute pancreatitis

In patients with acute pancreatitis, up to 20% of all deaths occur prior to admission to hospital. In these cases, acute lung injury seems to be the predominant cause of death. Acute lung injury is microscopically characterized by an initial exudative phase during day 1–3 with a diffuse alveolar damage, type I pneumocyte necrosis, and influx of inflammatory cells and fluid. This is followed by a proliferative phase from about days 3–7 with lung repair, type II pneumocyte hyperplasia and fibroblast proliferation.

Underlying pathophysiological mechanisms for ALI includes a variety of derangements of the normal homeostasis, including pulmonary endothelial and epithelial barrier dysfunction. In addition, neutrophils, monocytes, and...
macrophages, being present both prior to “challenge” and recruited at different phases by chemokines like interleukin-8 (IL-8) and monocyte chemoattractant protein (MCP)-1, become activated (Fig. 1). Pancreatitis-associated ALI is further related to specific effects on pancreatic enzymes like proteases and phospholipase A₂. Increased serum concentrations of phospholipase A₂ has been demonstrated in severe acute pancreatitis and correlates with the extent of pulmonary complications and lung injury scores.¹⁷⁻¹⁹

In this review we will focus on different pathophysiological mechanisms and potential interventions for the acute lung injury associated with acute pancreatitis. The majority of the studies referred to in this review are experimental studies in different animal models of acute pancreatitis.

Neutrophils

Induction of pancreatitis causes a prominent assembly of neutrophils within the capillaries of the lungs with at the time no apparent inflammatory cell infiltration noted in the pancreas. Neutrophils are the earliest immune cells to be recruited to the site of injury or inflammation and are considered to play a key role in the progression of ALI and ARDS, as activation and transmigration of neutrophils is a hallmark event in the progression of ALI and ARDS.²⁰⁻²³ The neutrophil sequestration to the lungs activates oxygen radicals, arachidonic acid metabolism as well as production of proteolytic and chemotactic substances in neutrophils, leading to pulmonary endothelial injury. In addition, there is evidence for interactions between granulocytes, endothelium, and endothelium-derived mediator substances, which together can amplify the lung injury. Ultimately, lung injury appears to result from local endothelial cell injury secondary to neutrophil-generated oxygen products that may be myeloperoxidase (MPO) dependent.²⁴ Since neutrophils accumulate in the lungs during ARDS and are also present in the lungs of cerulein-induced pancreatitis in rats, it is likely that they play an equivalent crucial role in pancreatitis-associated ALI.²⁰

During later phases, severe alterations of endothelial cells, including interstitial edema formation and thickening of the alveolar gas exchange barrier, are observed. Lung edema, endothelial and epithelial injuries are accompanied by an influx of neutrophils into the interstitium and bronchoalveolar space.

Proof for the importance of neutrophils in ALI comes both from clinical data and animal models. In patients with ARDS following sepsis, the percentage of BAL polymorphonuclear leukocytes (PMN) was higher in patients who died than in those who survived.²⁵ Prominent leukocyte margination in pulmonary microvessels and edematous thickening of alveolar septa have been reported in an experimental model of acute hemorrhagic pancreatitis in sheep. At later time-intervals, a marked hemorrhagic destruction of the lungs occurred with a complete loss of the alveolar architecture.²² Furthermore, severe endothelial cell destruction and marked pulmonary edema was found early after induction of hemorrhagic pancreatitis in rats.²³

Figure 1 The alveolar—capillary barrier is formed by two separate barriers, the microvascular endothelium and the alveolar epithelium. The early phase of acute lung injury is characterized by the increased permeability of the alveolar—capillary barrier. A complex network of cytokines and other pro-inflammatory compounds initiate and amplify the inflammatory response in acute lung injury. Cytokines such as TNF-α and IL-1, secreted by lung macrophages result in a substantial amplification of cytokine/chemokine expression by structural cells in the lung microenvironment. Cytotoxic products (e.g., reactive oxygen and proteases) are secreted by the leukocytes recruited to the lung (e.g., neutrophils and exudate macrophages).
In a model of acute necrotizing pancreatitis in rats, antibody-depletion of peripheral neutrophils, as well as blockage of neutrophil adherence functions using anti-CD18 antibodies, improved the survival rate and significantly decreased histological changes.\textsuperscript{26} Administration of anti-neutrophil serum in an experimental diet-induced pancreatitis model, using choline deficient ethionine, prevented the microvascular permeability and MPO rise in the lungs.\textsuperscript{27} The same findings were observed in caerulein-induced pancreatitis in rats.\textsuperscript{24} 

Intercellular adhesion molecule-1 (ICAM-1) plays an important role in the neutrophil-mediated pro-inflammatory response in pancreatitis and pancreatitis-associated lung injury. In addition, ICAM-1 and neutrophil-independent events also contribute to the development of pancreatitis and lung injury.\textsuperscript{28,29} Heat shock protein 72 (Hsp72) is over-expressed in bronchial epithelium, alveolar macrophages, infiltrating neutrophils and blood vessels in acute pancreatitis. It has been suggested that Hsp72 induction is mediated by neutrophil infiltration into the lungs via P-selectin.\textsuperscript{30} 

In conclusion, neutrophils, interacting with ICAM-1, play an important role in regulating the severity of pancreatitis-associated ALI.

**Macrophages**

Activation of different cell populations contributes to the generation of systemic inflammatory mediators. In particular, peritoneal macrophages, alveolar macrophages and Kupffer cells, activated during different stages of severe acute pancreatitis, have been implicated to contribute to disease progression.\textsuperscript{31–34} Macrophages can be activated depending on microenvironment through different activation pathways, resulting in marked phenotypic heterogeneity.\textsuperscript{35,36} 

In vitro activation of macrophages occurs after treatment with supernatants from rat pancreatic acinar cell cultures incubated with cerulein.\textsuperscript{37} Similarly, ascitic fluid from rats with experimentally induced pancreatitis activates macrophages in vitro.\textsuperscript{38,39} These results indicate that mediators released during acute pancreatitis have the capability to activate macrophages. Activated macrophages generate systemic cytokine and inflammatory mediators.\textsuperscript{40} 

Several macrophage populations get involved in the course of the disease. Macrophages as the main source for the production of inflammatory mediators, amplify the immune response during acute pancreatitis progression.

**Peritoneal macrophages**

Peritoneal macrophages are exposed to profound pro-inflammatory environment of ascitic fluid secreted by the pancreas and become activated. This activation may be of particular importance as inflammatory mediators released by these macrophages into the peritoneal cavity could easily reach the circulation, thus contributing to the systemic inflammatory response associated with acute pancreatitis.\textsuperscript{41} 

Release of IL-1β and TNF-α by peritoneal macrophages during the early stages of AP induce a cascade of additional inflammatory cytokines, activation of neutrophils, and induction of a net pro-inflammatory response. Cytokine neutralization has no beneficial effect on the degree of pancreatitis, however, it attenuates the systemic stress response and is associated with a modest decrease in mortality.\textsuperscript{42} Abnormal trypsin activation in the pancreas stimulates production of cytokines in rat peritoneal macrophages and injection of trypsin into the peritoneal cavity induces lung injury.\textsuperscript{43} The contribution of peritoneal macrophage-derived mediators to the toxicity of ascitic fluid was demonstrated when peritoneal lavage was carried out before the induction of pancreatitis in order to remove peritoneal macrophages.\textsuperscript{44} 

Deactivation of the activated pancreatic macrophages, as the major source of TNF-α, in the early course of AP increases survival and decreases the severity of disease. It has been suggested that activation of NF-κB and p38 MAPK in monocytes/macrophages from animals with AP might play a role in transcription and biosynthesis of TNF-α and IL-6.\textsuperscript{45,46} Animal experiments have indicated that sterile cytokine-depleted ascites from AP can stimulate in vitro cytokines production from macrophages derived from the spleen and lungs, and induce systemic cytokine production in vivo.\textsuperscript{47} 

By depleting peritoneal macrophages using liposome encapsulated dichloromethylene biphasphonate, the inflammation was extended from the pancreas to the peritoneal cavity and subsequently induced SIRS, lung injury and multiple organ failure. This somewhat contradictory finding could imply a possibility of therapeutic modification of peritoneal macrophages as a new therapeutic approach in patients with AP.\textsuperscript{48,49}

**Kupffer cells**

Another population of macrophages involved in the pathogenesis of acute pancreatitis is Kupffer cells. They are the most abundant mononuclear phagocytes in the body and a predominant source of inflammatory cytokines released into the systemic circulation. Kupffer cells can e.g. interact with mediators released by a damaged pancreas or present in ascitic fluid before they become “diluted” in the systemic circulation.\textsuperscript{50} Pancreatic blood, but not intestinal blood, plays a key role in liver activation during experimental acute pancreatitis.\textsuperscript{51} Once the inflammatory mediators reach the liver, Kupffer cells become activated and amplify their release of cytokines into the blood stream and thus contribute to the systemic manifestation of AP. Activated Kupffer cells release e.g. immune-regulatory and inflammatory cytokines, reactive nitrogen intermediates (RNI), reactive oxygen intermediates (ROI), and hydrogen peroxide, all playing significant roles in the progression of pancreatic inflammation into a systemic process.\textsuperscript{52} TNF-α triggers the early events in AP and as pancreatitis progresses, it reaches the liver, further manifesting the disease outcome by causing a profound cytokine release.\textsuperscript{53} The observation that the systemic manifestation of AP is more severe as compared to graft pancreatitis (as the mediators released by the pancreas in graft pancreatitis are sent directly through the iliac vein) implicates that the liver plays an active role in the development of lung injury secondary to AP.\textsuperscript{54} This observation has been further...
confirmed by preventing the passage of blood from pancreas to the liver.40,55

In vitro analysis of Kupffer cell activity revealed that they also become activated by pancreatic enzymes.52,56 Inhibition of Kupffer cell activity, by gadolinium chloride administration before the induction of acute pancreatitis, results in reduced levels of circulating cytokines and prevents the pathological injury in the lungs, but not the pancreatic damage.55,57–61 These results indicate that liver involvement and activation of hepatic macrophages amplify the inflammatory signals induced by the pancreas and subsequent secondary systemic organ dysfunction. Interestingly, the liver itself is not affected by this process and liver damage is not evident during the early stages of pancreatitis.58,62

Alveolar macrophages

The third family of macrophages involved in the progression of acute pancreatitis is alveolar macrophages. It has been postulated that alveolar macrophages (AM) are involved in the development of acute local disorders as a consequence of extra-pulmonary stimuli like acute pancreatitis, peritonitis, or trauma. Alveolar macrophages have the capacity to secrete a vast number of chemokines, cytokines, growth factors and reactive oxygen and nitrogen species. Hence, they possess multiple pro- and anti-inflammatory roles in the respiratory tract. The activation of the alveolar macrophages leads to the recruitment of leukocytes from the circulation, including monocytes, neutrophils and T lymphocytes.

Alveolar and interstitial macrophages play distinct roles in the acute lung injury associated with acute pancreatitis. Alveolar macrophages promote an early inflammatory response, whereas interstitial macrophages appear to have a protective role to resolve the inflammation.63 Increased NO synthesis related to induction of iNOS in alveolar macrophages has been suggested to contribute to the acute lung injury secondary to pancreatitis.54,64 The use of phospholipase A2 (PLA2) inhibitors indicate that this enzyme could be involved in the activation of alveolar macrophages and generation of nitric oxide.65 PLA2 has further been shown to regulate the cytokine production by monocytes/macrophages as well as phagocytosis and superoxide (O2−) generation by neutrophils.66

Inhibition of NF-κB activation may reverse the lung injury in acute necrotizing pancreatitis by inhibiting the release of inflammatory mediators by alveolar macrophages.61 Neutrophil recruitment to the lungs during AP is in part mediated by chemotactic mediators (TNF-α and MIP-2) released by activated alveolar macrophages.64 In AP, endothelial cells, neutrophils and macrophages release platelet activating factor (PAF), which has been implicated as a key mediator in the progression of AP, leading to complications and high mortality rates.

Macrophages polarization

Peritoneal macrophages frequently show a classical pro-inflammatory M1 phenotype in AP, characterized by high expression of TNF-α, IL-1β, IL-6, iNOS, COX-2 and lack of changes in the mannose receptor.35,67,68

A recent study indicates that peritoneal macrophages could be reprogrammed in vitro to activated (M2) macrophages by the Th2 cytokines IL-4 and IL-13, thus opening the possibility for therapeutic modulation of macrophage activation in the treatment of AP. M2a macrophages fail to produce NO and to present antigens to T cells.67,69,70

Increased expression of iNOS71 and TLR450 were found in peripheral blood monocytes from patients with severe acute pancreatitis, indicating elevated levels of activated monocytes in the circulation.

Interventions

Initial treatment strategies have focused on the inhibition of macrophages. Administration of the macrophage-pacifying compound CNI-1493, prior to the induction of severe acute pancreatitis in rats results in an increased survival and reduced disease progression manifested by reduced levels of circulating enzymes, cytokines, as well as transaminases.72,73 Macrophage ablation also confers a protective effect on disease progression in a mice model of adenoviral-induced pancreatitis, although reduced levels of the potentially protective cytokine IL-10 was observed.74 In additional experimental models, the use of gadolinium chloride to inhibit Kupffer cells,40,52 liposome encapsulated dichloromethylene-diphosphonate to inhibit peritoneal macrophages,50 or PAF antagonists to block the activation of alveolar macrophages,75 resulted in modulation of the systemic inflammatory response. Unfortunately, in these studies the macrophage inhibitors were administered before the induction of pancreatitis and the translation of these findings into clinical practice is thereby difficult.

Moreover, the extensive time required for efficient macrophage ablation or inhibition of their activity constitutes a limiting factor to experimental studies. Consequently, other approaches have been evaluated based on the ability of macrophages to modify their phenotype. An interesting approach is to polarize macrophages ex vivo toward M2 cells and subsequently transfer these cells into animals with acute pancreatitis.76 This approach results in a reduction of histological score and in levels of circulating amylose. However, the long time required to obtain polarized macrophages remain and may limit its approach as a therapeutical strategy.

Together, these studies reveal that the development of lung injury is accompanied by dramatic changes in macrophages. The above referred studies demonstrate a role for macrophages in determining the severity of the acute lung injury, opening a novel area of investigation to identify new therapies in the treatment of patients with acute lung injury.

Mast cells

Mast cells are regarded as potentially important in the initiation and/or amplification of an acute inflammatory response.77 Activated mast cells induced by pancreatitis appear to play a critical role in the initiation of pancreatitis-associated lung injury. This further involves
endothelial barrier dysfunction in both the pancreas and extra-pancreatic organs/tissues, particularly in the lungs and colon. In a rat model of intraductal administration of sodium taurodeoxycholate, the permeability of the endothelial barrier significantly increased 6 h after induction of pancreatitis. However, administration of the mast cell stabilizer cromolyn prevented against pancreatitis-induced endothelial barrier compromise in the lungs and systemic histamine levels. In addition, it prevented against a decrease in circulating PECAM-1 positive neutrophils and monocytes/macrophages. The number of ICAM-1 and PECAM-1 positive pulmonary neutrophils and monocytes/macrophages decreased 6 h after induction of pancreatitis. This indicates that mast cells may play a critical role in the activation of leukocytes during the initiation of pancreatitis-associated lung injury by altering adhesion molecule expression. Pancreatic mast cells play an important role in triggering the local and systemic inflammatory response during the early stages of acute pancreatitis. In contrast, pulmonary mast cells are not directly involved in the inflammatory response related to pancreatic damage.

Cytokines and chemokines

In the pathogenesis of respiratory complications following AP, cytokines and chemokines, in particular IL-1β, IL-6, IL-8, IL-18 and TNF-α, play major roles.

TNF-α and IL-1β

Intravenous administration of pancreatic ascites to healthy rats increased the leukocyte number in BAL and induced lung injury, whereas the severity of pancreatitis was similar in both groups.97 Whereas the severity of pancreatitis was similar in both groups.97

Macrophage migration inhibitory factor (MIF), a pro-inflammatory cytokine released by macrophages and lymphocytes, is emerging as an important factor in the pathogenesis of AP. Pre-treatment with anti-MIF antibodies improved the survival of rats with AP.95

The ratio between pro- and anti-inflammatory mediators can be of importance in predicting the severity of pancreatitis and pancreatitis-associated lung injury. The pro-inflammatory cytokine IL-18 has been suggested to be used as a clinical marker in screening tests. AP patients with IL-18 levels exceeding 650 pg/ml are likely to develop pulmonary dysfunction.96 In contrast, the anti-inflammatory cytokine IL-10 counteracts the release of pro-inflammatory cytokines from macrophages. The lung injury following diet-induced pancreatitis was more pronounced in IL-10 knockout mice than in wild-type mice, whereas the severity of pancreatitis was similar in both groups.97

Substance P

The neuropeptide substance P is a pro-inflammatory mediator involved in pancreatitis. The biological properties of substance P are mainly (but not exclusively) mediated via the neurokinin 1 receptor (NK-1R). During acute inflammation substance P induces chemokine release from macrophages infiltrating local and distant damaged tissues. It induces selective chemokine production in murine RAW264.7 macrophage cells, as well as in primary macrophages.98 Mice lacking NK-1R are protected against cerulein-induced pancreatitis and associated lung injury.99–101 Similar findings have been observed in mice treated with the specific NK-1R antagonist, CP-96345.
Pulmonary microvascular permeability was also reduced as a result of CP-96345 treatment.102 Deletion of the substance P gene precursor preprotachykinin-A (PPT-A) has a protective effect against acute pancreatitis-associated lung injury, with a partial protection against local pancreatic damage.103 Associations between substance P and the pro-inflammatory gasotransmitter hydrogen sulphide (H₂S) have been demonstrated in pulmonary inflammation and in acute pancreatitis.104 Prophylactic or therapeutic administration of α-Propargylglycine (PAG), an irreversible inhibitor of the H₂S synthesizing enzyme CSE, significantly reduced H₂S and substance P levels in plasma, pancreas and lungs in a cerulein-induced acute pancreatitis model. In addition, PPT-A and NK-1R mRNA expression were reduced in both pancreas and lungs. These results suggest that the pro-inflammatory effects of H₂S in acute pancreatitis may be mediated via the substance P/NK-1R pathway.105 Ghrelin, an anti-catabolic hormone, can significantly down-regulate pulmonary substance P expression and attenuate the severity of acute lung injury induced by AP.106

**Platelet activating factor**

Platelet-activating factor (PAF) is a pro-inflammatory phospholipid that promotes recruitment and activation of inflammatory cells, and is further involved in bronchoconstriction and in altering microvascular permeability. During pancreatitis, the levels of PAF in the pancreas increase with disease progression and has been implicated to play a role in the systemic involvement and associated lung injury.107–109 Administration of the PAF degrading enzyme, PAF-acetylhydrolase, after initiation of pancreatic injury, prevents the development of pancreatitis-associated lung injury.109 Several PAF antagonists have been evaluated in experimental models and clinical trials for the treatment of acute pancreatitis and prevention of organ failure.75,108,110,111 PAF antagonist (TCV-309) attenuated hyperactivity of bronchoalveolar macrophages and pancreatitis-associated lung injury in an experimental acute pancreatitis model. The preventive effects were associated with reduced levels of CINC in the circulation as well as its local expression in the lungs.112 Another PAF antagonist, BN 52021, significantly prevented hyperamylasemia due to experimental pancreatitis, reduced necrotic and inflammatory changes in the pancreas and improved survival rate.113 The systemic inflammatory response in a murine model of mild acute pancreatitis was reduced after treatment with the PAF antagonist lexipafant.114 Lexipafant is one of the few interventions which has led to clinical trials. Lexipafant treatment significantly reduced serum IL-8 and IL-6 levels, but had no effect on serum C-reactive protein in a double-blind, placebo-controlled study (n = 83).115 However, in a larger randomized, double-blind, placebo controlled, multicenter trial (n = 290), lexipafant had no preventive effects on organ failure in acute pancreatitis, suggesting that PAF antagonism alone is not sufficient to improve the systemic inflammatory response syndrome SIRS in severe acute pancreatitis.116

**Matrix metalloproteinases**

Pancreatitis results in increased local and distant matrix metalloproteinase (MMP) activity that has been suggested to contribute to the systemic complications and pulmonary injury. Treatment with the MMP inhibitor Batimistat (BB-94) reduced neutrophil transmigration and alveolar-capillary leakage in pancreatitis-associated lung injury. In addition, the MMP inhibitor reduced the pancreatic and lung injury and improved survival following experimental acute pancreatitis. Further evaluation showed that MMP-9 was highly expressed in the lungs and in neutrophil supernatants, suggesting an important role of neutrophil-derived MMP-9 in the pathogenesis of pancreatitis-associated lung injury.117,118 Specific inhibition of MMP-9 activity with doxycycline has demonstrated similar effects.119

**Poly (ADP-ribose) polymerase-1**

Poly (ADP-ribose) polymerase-1 (PARP-1) is a nuclear DNA-binding protein involved in apoptosis, cell necrosis and organ failure in various inflammatory conditions. Genetic deletion or pharmacological inhibition of PARP-1 attenuates the severity of acute pancreatitis and pancreatitis-associated lung injury. This effect may be due to prevention of pancreatic cell necrosis mediated by reactive oxygen species (ROS) released via the neutrophil enzyme NADPH oxidase.120,121

**NF-κB**

NF-κB is a central transcription factor that controls the expression of multiple inflammatory genes, such as TNF-α, IL-6, IL-8 and inducible nitric oxide synthase (iNOS). Overexpression of these factors can cause damage to pancreatic and extra-pancreatic tissues in AP. A correlation between NF-κB activation, serum amylase, reactive oxygen species (ROS) levels and pancreatic and pulmonary tissue damage has been implicated in the pathogenesis of AP. Inhibition of NF-κB activation using PDTC (pyrroolidine dithiocarbamate), prevented pancreatic damage and reactive oxygen species (ROS) production in rat AP. PDTC treatment further reduced the expression of iNOS mRNA and effectively improved the severity of lung injury.122,123 Sphingosine-1-phosphate (S1P), a biological active lipid, reduces the NF-κB activation of alveolar macrophages, which leads to prevention of pancreatitis-associated lung injury.124

**Pancreatitis-associated proteins**

Pancreatitis-associated proteins (PAPs) belong to the regenerating (Reg) secretory protein family, which is strongly induced during acute pancreatitis. PAP has been proposed as a potential marker for disease identification and classification of severity of ongoing acute pancreatitis in rats.125 Although originally identified during AP, PAPs have been reported in e.g. Crohn’s disease, inflammatory bowel disease, liver injury, neuronal, ovarian, and cardiac tissue damage.126,127 Recent investigations suggest that PAP2 is a potential mediator of early inflammation in AP,
acting specifically by orchestrating the macrophage inflammatory response through putative a PAP2 receptor. It has also been suggested that PAP proteins mediate the systemic inflammation response of pancreatitis.\textsuperscript{128}

Further experimental interventions

Several studies have evaluated the beneficial effects of the somatostatin analog octreotide and the flavone and anti-inflammatory agent baicalein in experimental severe acute pancreatitis rat models. These studies suggest that both octreotide and baicalein effectively decrease SAP complications, protect against multiple organ injury and improve the survival rate in SAP. The protective effects were further accompanied with reduced serum levels of TNF-\textsubscript{\alpha}.\textsuperscript{129–131}

Erythropoietin (EPO) administration prevented the changes of ALI following acute necrotizing pancreatitis in rats and decreased the levels of pro-inflammatory cytokines and oxidative stress markers. EPO further inhibited neutrophil accumulation, maintained microvascular endothelial cell integrity and reduced oxidative stress-associated lipid peroxidation.\textsuperscript{132}

Pentoxifylline, a nonspecific phosphodiesterase inhibitor, was recently shown to have an immune modulating role including attenuation of pro-inflammatory neutrophil functions. Pentoxifylline significantly attenuated histological evidence of pulmonary injury, pulmonary neutrophil activity, and pro-inflammatory signaling in bile-induced AP.\textsuperscript{133}

Several other interventions such as rosiglitazone, emodin, edaravone, lidocaine, propentofylline, losartan, resveratrol and exogenous leptin have shown protective effects on the acute lung injury due to acute pancreatitis.\textsuperscript{134–141}

Clinical studies

Several prospective randomized clinical trials have targeted pancreatic injury to treat acute pancreatitis. Protease inhibitors, such as aprotinin and gabexate mesylate, have not shown benefit in patients with severe acute pancreatitis.\textsuperscript{142–144} Controlled clinical trials have failed to demonstrate the therapeutic efficacy of antioxidants.\textsuperscript{145} Platelet-activating factor receptor antagonist, lelipafant, reduced the severity score for all organ dysfunction in patients with severe acute pancreatitis. However, another study reported no efficacy of lelipafant.\textsuperscript{146} The only treatment that improved acute pancreatitis-associated lung injury was thoracic duct drainage, but this treatment has not been tested in a large clinical trial.\textsuperscript{146}

Conclusion

Mechanisms involved in acute lung injury (ALI) associated with severe acute pancreatitis are complex and probably not significantly different from the ALI caused by other underlying disorders. We still lack effective treatment directed at underlying pathophysiological mechanisms and treatment in general has to be considered as merely being organ supportive. In order to improve results and render novel types of intervention, increased knowledge on mechanisms including involved cells, chemokines, cytokines and stromal components, during different phases of ALI, may render novel and more targeted types of therapy, probably to be used in a multimodal fashion.

Conflict of interest statement

Hereby, all the authors certify no conflict of interest in the collection and interpretation of data, in the writing of the manuscript, and in the decision to submit the manuscript for evaluation by Respiratory Medicine.

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H. Akbarshahi et al.


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