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Review article

Breakdown of bioenergetics evoked by mitochondrial damage in acute pancreatitis: Mechanisms and consequences

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ABSTRACT

Acute pancreatitis is a severe inflammatory disease with unacceptably high mortality and without specific therapy. Clinical studies revealed that energy supplementation of patients via enteral feeding decreases systemic infections, multi-organ failure and mortality. These clinical observations have been supported by *in vitro* and *in vivo* experimental studies which showed that the most common pancreatitis inducing factors, such as bile acids, ethanol and non-oxidative ethanol metabolites induce intracellular ATP depletion and mitochondrial damage both in pancreatic acinar and ductal cells. Notably, the *in vitro* supplementation of ATP prevented the cellular damage and restored cell functions in both cell types. These observations suggest that either prevention of mitochondrial damage or restoration of intracellular ATP level might provide therapeutical benefits.

Introduction

Acute pancreatitis (AP) was the most common cause of hospitalization for non-malignant gastrointestinal diseases in the USA in 2012 leading to ~270,000 hospital admission/year with an estimated annual cost of ~2.5 billion dollars [1,2]. The majority of the cases are mild (~80%), however in the severe form (when multi-organ failure is persistent > 48 h), the mortality rate can reach as much as 40% [3]. Although AP is a severe problem, no specific pharmacological therapy is currently available. Therefore, there is a pressing economic and clinical need for developing new therapies for the treatment of AP.

During the pathogenesis of AP zymogen granules fuse with intracellular lysosomes and form autophagic vacuoles in the pancreatic acinar cells in response to direct cellular stress caused by different toxic agents (such as bile acids, ethanol and its metabolites). In the fused vacuoles cathepsin B (a lysosomal enzyme) converts trypsinogen to trypsin leading to cellular autodigestion [4]. Gaiser et al. in an elegant study used genetically engineered mice that conditionally express an endogenously activated trypsinogen within pancreatic acinar cells [5]. With this approach they provided direct evidence that intra-acinar activation of trypsinogen is sufficient to initiate AP without the activation of the immune system. They also showed that the dominant form of cell death in this model was apoptosis in the early phase of acute pancreatitis. However, necrosis, another form of cell death, is present in AP as well [6], which have been shown to correlate directly with the severity of experimental AP [7]. Notably, apoptosis is in inverse correlation with the severity, without the activation of the immune system limiting the pancreatic damage [5]. It is well documented that mitochondria play a central role in the differentiation between apoptosis and necrosis [6], since the loss of mitochondrial membrane potential ($(\Delta\Psi)_m$) and the consequent drop in the cellular ATP level promotes necrosis, whereas the release of cytochrome c from the intermembrane space promotes apoptosis. In this review we will focus on the mechanism and consequences of mitochondrial damage and breakdown of cellular bioenergetics in AP.

From the bed: clinical aspects of energy demand in acute pancreatitis

The current IAP/APA guideline for the management of AP involves fluid resuscitation, intensive care management and

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highlights the importance of nutritional support in predicted severe AP [3]. According to the guideline enteral tube feeding should be the primary therapy in patients with predicted severe AP who require nutritional support. In recent years, several meta-analyses and clinical trials confirmed that enteral nutrition via nasojejunal tube feeding decreases systemic infections, multi-organ failure, need for surgical intervention, and mortality, as compared with total parenteral nutrition in patients with predicted severe AP [8,9]. The explanation for these findings embraces the facts that prolonged parenteral feeding induces atrophy and increased permeability of the gut mucosa, hypomotility of the gut due to the lack of stimulation and overgrowth of abnormal intestinal flora [10]. These pathophysiological mechanisms can lead to bacterial translocation and superinfection of the inflamed pancreatic tissue. However, a recent randomized multicentric clinical study challenged these arguments. Bakker et al. compared the early (within 24 h) nasoenteric tube feeding with an oral diet at 72 h after presentation to the emergency department in patients with AP [11]. Their primary end points were major infection (infected pancreatic necrosis, bacteremia, or pneumonia) or death during 6 months of follow-up. This study found no significant difference neither in the rate of infection, nor in the rate of death, questioning the importance of the above described beneficial effects of enteral feeding. One possible explanation for the negative outcome might be that although enteral feeding was started within 24 h, the calorie intake reached the optimal 25 kcal/kg/day only on the third day after admission in accordance with the ESPEN guidelines on parenteral nutrition [12]. However, the pathophysiological changes involving mitochondrial damage and ATP depletion are crucial early steps of the pancreatic injury during the pathogenesis of AP [13]. These facts suggest that the calorie intake in these patients should be increased sooner to compensate for the increased energy demand of AP patients.

To the bench: mitochondrial injury and intracellular ATP depletion in acute pancreatitis

Mitochondrial damage and ATP depletion have been highlighted as one of the crucial events in the development of AP [6,14,15] (Fig. 1). In an early study Nordback et al. demonstrated that the intracellular ATP levels decrease during the early phase of different experimental AP models Changes in high-energy phosphate metabolism and cell morphology in four models of acute experimental pancreatitis [16]. The most common pancreatitis inducing factors, such as bile acids, ethanol and non-oxidative ethanol metabolites cause mitochondrial damage via a complex mechanism. These agents have been shown to release Ca^{2+} from the endoplasmic reticulum (ER) and induce extracellular Ca^{2+} influx. Since mitochondria act as cellular Ca^{2+} buffers, the sustained increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), leads to mitochondrial Ca^{2+} overload and to decreased intracellular ATP ($(ATP)_i$) production. ATP is necessary for the activity of the sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA) and plasma membrane Ca^{2+}-ATPase (PMCA) that remove Ca^{2+} from the cytosol. Decrease of $(ATP)_i$ impairs the cellular Ca^{2+} clearance and therefore contribute to the maintenance of sustained Ca^{2+} elevation. Prolonged mitochondrial Ca^{2+} overload can lead to the opening of the mitochondrial membrane permeability transition pore (MPTP) across the inner and outer membranes of mitochondria, resulting in an increased permeability of the mitochondrial membranes to molecules and ions with molecular mass less than 1.5 kDa, including protons and water [17]. Another possible way of mitochondrial membrane permeabilization is the mitochondrial outer membrane permeabilization (MOMP), which is supposed to be a crucial event during apoptosis and causing the release of proapoptotic factors

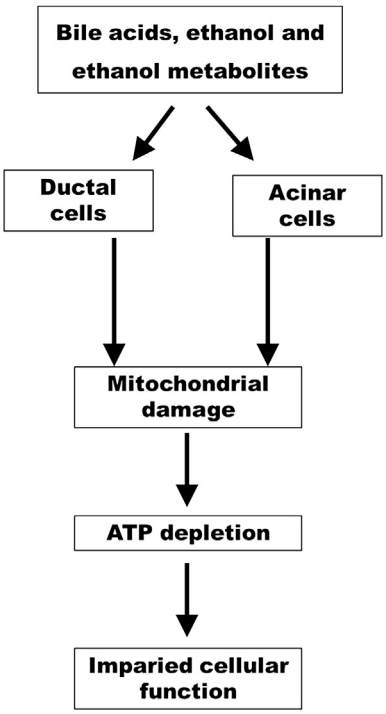

Fig. 1. Central role of mitochondrial damage in acute pancreatitis. Bile acids, ethanol and its metabolites induce mitochondrial damage both in pancreatic acinar and ductal cells. The cellular ATP level consequently decreases, which impairs a plethora of cellular functions, including cellular Ca^{2+} signaling and bicarbonate secretion in pancreatic ductal cells. The impaired cell function will lead to the development of acute pancreatitis.

from the mitochondrial intermembrane space to the cytosol [18]. The increased membrane permeability results in mitochondrial swelling and dissipation of $(\Delta\Psi)_m$, with a consequent drop in ATP production [17,19,20]. Sustained elevations of $[Ca^{2+}]_i$ and the resulting mitochondrial damage lead to a vicious cycle, which in turn triggers cell necrosis [21,22].

The role of mitochondrial injury and ATP depletion in bile acid-induced pancreatic damage

Bile acids were shown to induce Ca^{2+} release from the ER and acidic Ca^{2+} stores via inositol trisphosphate receptors (IP_3R) and ryanodine receptors (RyR) activation [23], inhibit SERCA pump activity and decrease the level of $(ATP)_i$ in pancreatic acinar cells [24,25]. On the other hand Booth et al. demonstrated that bile acids induce an increase in the intracellular and mitochondrial reactive oxygen species (ROS) production [26]. The increased ROS production promoted apoptosis and decreased necrosis. In isolated guinea pig pancreatic ductal epithelial cells (PDEC) the non-conjugated bile acid, chenodeoxycholate (CDC) induced toxic sustained Ca^{2+} increase and inhibited the activity of basolateral Na^+/H^+ exchanger, Na^+/HCO_3^- cotransporter and luminal Cl^-/HCO_3^- exchanger [27]. In these series of experiments, loading with BAPTA did not prevent the inhibitory effect of CDC on HCO_3^- secretion [28]. We also showed that CDC treatment induces morphological damage of the mitochondria and consequent $(ATP)_i$ depletion in the ductal cells [25]. Accordingly, loading pancreatic ducts with BAPTA failed to prevent the CDC-induced mitochondrial damage suggesting a Ca^{2+}-independent mechanism underlying the observed mitochondrial damage in response to CDC. In addition, ATP depletion was shown to directly inhibit pancreatic ductal HCO_3^- secretion [28].

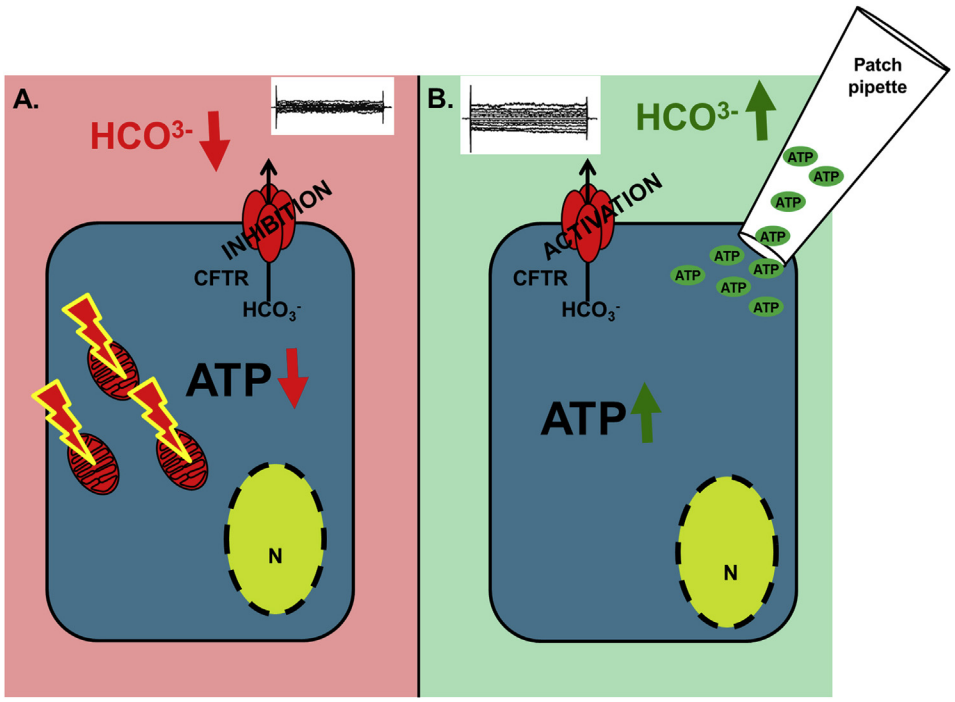

Fig. 2. ATP supplementation restores pancreatic ductal secretion *in vitro*. A. During the pathogenesis of AP different toxic agents damage the mitochondria of the pancreatic ductal epithelial cells resulting in a consequent drop in the intracellular ATP level. Due to the impaired ATP level several cellular functions get disturbed, including CFTR activity and bicarbonate secretion. B. *In vitro* supplementation of the intracellular ATP level via a patch pipette restores CFTR activity in pancreatic ductal cells.

The role of mitochondrial injury and ATP depletion in alcohol-induced pancreatic damage

In pancreatic acinar cells, the non-oxidative ethanol metabolite fatty acid ethyl esters (FAEE) and fatty acids (FA) were found to induce a sustained elevation of $[Ca^{2+}]_i$, leading to necrosis similarly to bile acids [29–31]. Criddle et al. have found that high concentration of palmitoleic acid (POA) depolarizes the $(\Delta\Psi)_m$. Furthermore, preincubation of acinar cells with BAPTA abolished the loss of $(\Delta\Psi)_m$, but NADH fluorescence was still decreased by POA under these conditions [30], which suggest that non-oxidative ethanol metabolites can inhibit mitochondrial functions independently of $[Ca^{2+}]_i$ elevation. On the other hand, high concentrations of ethanol (100 mM) and POA (200 μM) inhibited the activities of the apical SLC26 Cl^-/HCO_3^- exchanger and CFTR Cl^- channel and decreased the HCO_3^- secretion in PDEC (Maleth et al., 2015). We also showed that ethanol and POA at high concentrations induces sustained $[Ca^{2+}]_i$ elevation by releasing Ca^{2+} from the ER via IP_3R and RyR activation and gadolinium-sensitive extracellular Ca^{2+} influx. BAPTA loading completely abolished the inhibitory effects of ethanol and POA, suggesting that the inhibition was mediated by sustained $[Ca^{2+}]_i$ elevation [32]. We also observed decreased $(ATP)_i$ levels and the loss of $(\Delta\Psi)_m$ during the administration of high concentration of ethanol or POA.

Mitochondrial damage in in vivo acute pancreatitis studies

In an experimental AP model, Biczó et al. showed that treatment with large doses of L-lysine caused mitochondrial damage, which happened earlier than the activation of trypsinogen or NF-κB [33]. These results suggest that L-lysine may directly damage mitochondria, which seems to be the initiating event in this pancreatitis model. Impaired mitochondrial function and mitochondrial damage have also been observed in other *in vivo* AP models. Halangk et al. demonstrated that, in cerulein-induced AP the mitochondrial oxidative phosphorylation and ATP production are drastically decreased in rat pancreatic acinar cells [34]. Moreover, they also showed that the opening of the MPTP plays a crucial role in this AP model [35].

From the bench: restoration of intracellular ATP in exocrine pancreatic cells

In vitro studies

The observations described above suggest that mitochondrial damage and the consequent ATP depletion are indeed a significant factors during the development of AP, therefore, restoration of the $(ATP)_i$ level should have beneficial effects. Supplementation of cellular ATP *in vitro* can be achieved by increasing the ATP concentration in patch pipette during whole-cell patch clamp recording. Using this approach we showed that intracellular administration of 5 mM ATP via the patch pipette diminished the inhibitory effect of POA on CFTR Cl^- current in isolated guinea pig PDECs [36] (Fig. 2.). Notably, Criddle et al. also demonstrated that ATP supplementation by the same approach prevents the transformation of the cellular oscillatory $[Ca^{2+}]_i$ signals to sustained, global Ca^{2+} elevation during POA administration [30]. In addition, the same beneficial effect of ATP restoration has been demonstrated by the same group on the TLC-S induced acinar cell damage and necrosis [26]. These results suggest that the restoration of the cellular energy level can be beneficial in AP, which can prevent the cellular dysfunction and cell damage.

In vivo studies

Although *in vitro* ATP restoration can be achieved, *in vivo* ATP delivery is more challenging. ATP is highly sensitive to enzymatic hydrolysis and has very poor cellular penetration; therefore, one of the key questions is how to deliver this chemical energy into the

cells. Both ATP-loaded liposomes and immunoliposomes were found to protect the myocardium against acute experimental myocardial infarction [37–39]. Verma et al. demonstrated that the intracoronary infusion of ATP loaded liposomes significantly reduced the extent of irreversible myocardial damage versus empty liposomes [40]. Moreover, they also showed that the infusion of encapsulated ATP significantly lowered the left ventricular end diastolic pressure [41]. Entrapped ATP also had protective effects against brain and liver damage in experimental ischemia models [42–46]. Laham et al. showed that intracarotidal administration of liposomal entrapped ATP greatly increased the number of tolerated episodes of brain ischemia [43]. Konno et al. showed that liposomal ATP delivery protected the liver from ischemic injury during hypovolemic shock [44]. Together, these results strongly suggest that encapsulation of ATP into liposomes is potentially effective in the prevention of cellular ischemia and therefore colloidal drug delivery systems may create a new therapeutic option in AP.

Summary and conclusions

Our brief review summarizes the recent advances in the role of compromised bioenergetics and mitochondrial damage in AP. As demonstrated the most common pancreatitis inducing agents, such as bile acids, or ethanol and its metabolites, both induce mitochondrial damage and cellular ATP depletion in pancreatic acinar and ductal cells. Based on these findings, we believe that prevention of mitochondrial injury and/or $(ATP)_i$ supplementation could have therapeutic consequences in the early phase of AP.

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