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Molecular Biology of Acute Pancreatitis

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1. Introduction

In the Acute Pancreatitis the pancreatic enzymes are activated locally, causing a tissue injury followed by a pancreatic inflammation, characterized by edema, leucocyte infiltration, hemorrhage and necrosis.

The etiology of the pancreatitis presents as major cause of this disease, the alcoholism and the gallstone. These risk factors are common in the actual society and are determining to the increase pancreatitis incidence.

Despite the default in the establishment of the initializing mechanism of the acute pancreatitis, experimental studies suggest a disturbance in the intracellular calcium levels as a first event of the process.

Following this idea, a duct obstruction or alcohol exposure could modify the calcium release, leading to a migration of exocytosis machinery from the apical area of the membrane to the basolateral area. The relocation of the enzyme vesicles and the release of the content in the inter membrane space are the starter of pancreatic auto-digestion.

Experimental pancreatitis studies have shown that the membrane permeability (disruption) of the acinar cell is crucial to the calcium disorders and consequent pathologic mechanisms, resulting in the release of cytoplasmic proteins. However, the understanding of the deficiency remains unclear, i.e., if it is a disturbance of the plasmatic membrane or a delay in the calcium-dependent mechanisms for cell membrane resealing.

Actually, the membrane exocytosis machinery can be disturbed under the exposition to different stimulation. It has been reported a blockade apical granule fusion after stimulus with cholecystokinin (CCK) and carbachol, for example.

The fusion of zymogen granules occurs in a very limited apical region of surface membrane to increase the effectiveness of pancreatic content delivering. This efficiency is due to the protein machinery that control the direct fusion of the zymogen granules with the membrane as well as the granule-to-granule fusion of the distant granules to the most apically located. This granule-granule fusion remains occurring despite of the apical releasing blockade and could be responsible to aggravate the basolateral exocytosis, the event possibly responsible by the pancreatitis starting.

The definition of initial cause of the acute pancreatitis is decisive to the control of late complication in this disease. However, the knowledge about the exact mechanism among a variety of etiologies still remains controversial.

Despite the alcoholism be often associated with a chronic pancreatitis, there are several reports of bouts of acute pancreatitis resulting of alcohol abuse. This could be due to the

association of the raised gut permeability with alcohol intake and the concomitant endotoxemia. In other view, the pancreatic stellate cells could be activated by the alcohol consumption. The role of these cells in pancreatic injury is similar to the liver injury during alcoholism, with the fibrosis establishment. Thence, the mechanism related to these cells is more evident in the chronic pancreatitis.

The molecular mechanism of biliary acute pancreatitis is Ca^{2+} mediated. Several authors suggested that the increase in bile acids preventing reuptake of Ca^{2+} by a PI3K-dependent mechanism.

The molecular mechanism of gallstone – associated pancreatitis seems to be simpler. The pancreatic duct obstruction confine the zymogen and lysosomal granules causing the condensing vacuoles and impeding the acinar exocytosis. Consequently, the trypsinogen is activated to trypsin and triggers the cascade of enzyme activation leading to the pancreatic injury.

Pancreatitis induced by hypertriglyceridemia is associated to amylase release and the cell injury due to the free acids released because its detergent properties.

2. Molecular biology of multiple organ failure during acute pancreatitis

The local and systemic complications during pancreatitis aggravate the prognostic of the disease. The morbidity and the mortality pancreatitis-associated occur due the systemic inflammation and the multiple organ dysfunctions, mainly lung, liver and kidney.

The intra and extra pancreatic events of the acute pancreatitis are responsible by the complexity of the disease. Initially, there is the local activation of the trypsinogen. Subsequently, the trypsine activates other enzymes those turn begin to digest the pancreatic tissue, whose content leaks into the abdominal cavity, causing cytokine release, activation of the immune system, coagulation and fibrinolysis.

The cytokines activates the inflammatory pathway, resulting in the increase of adhesion molecules, neutrophils infiltrate, production of Reactive Oxygen Species (ROS) and several inflammatory molecules such as Prostaglandin E2 (PGE2), Tromboxan A2 (TXA2), Platelet Activating Factor (PAF). On the other hand, the release of these mediators leads to Systemic Inflammation Response Syndrome (SIRS).

The ascitic fluid released in response to pancreatic inflammation could lead to activation of Kupffer Cells that will produce cytokines and others mediators, such protelytic enzymes, establishing the hepatic inflammation. The lung is often a target of these mediators and the dominant cause of mortality.

It has been shown that severe acute pancreatitis correlates with the incidence of hepatic injury. Although the liver is known to be a primary target of cytokines released in pancreatic blood, the liver itself releases inflammatory substances, such as reactive oxygen species, thereby leading to the injury of distant organs.

The oxidative stress is important in the early stages of the systemic inflammation that occurs in pancreatitis and the liver is a target of this event. Also, the liver is a source of oxidative stress. Multiple hepatic cells, including hepatocytes, Kupffer cells, stellate cells, endothelial cells can generate nitric oxide, superoxide, and peroxynitrite. On the other hand, these oxygen and nitrogen – derived species might lead to the early Hepatic Stellate Cells (HSC) activation, which leads to alteration in the extracellular matrix components and involves as much degradation as fibrogenesis. The extracellular matrix degradation could be responsible by the amplification of the inflammatory mediators release and the systemic

inflammation (Figure 1). Moreover, the oxidative and nitrative stress are responsible by the alteration in the mitochondrial respiration and the consequent apoptosis induction.

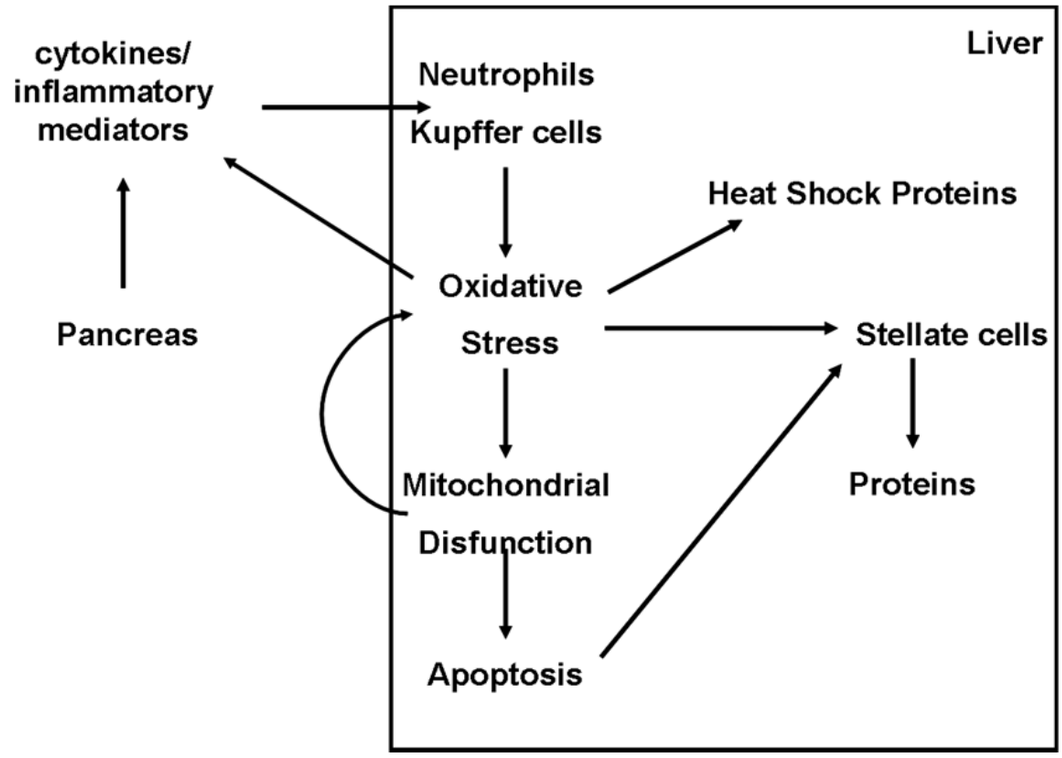

Fig. 1. The hepatic injury involved in the multiple organ failure during pancreatitis. The liver is both a source and target of the inflammatory mediators systemically released during pancreas inflammation. These mediators activate several hepatic cells causing oxidative stress, mitochondrial dysfunction and consequently apoptosis and synthesis of inflammatory proteins that will aggravate the involvement of distant organs.

3. Molecular signaling of the oxidative stress

Despite the initial cause of pancreatitis, the oxidative stress is the mainly contributing factor to the destruction of the pancreatic tissue. Moreover, the dysbalance redox participates of hepatocellular injury as well as the pulmonary lesion.

Among the effects of the pancreatitis in the liver it was demonstrate the reduction of oxygen consume by the mitochondria in animals that received samples of ascitic fluid from rats with acute pancreatitis.

Reactive oxygen species (ROS) activates necrosis and modify the production of cytokine and adhesion molecules. Furthermore, lipid peroxidation products are chemotactic and might lead to amplification of the inflammation process.

It is known that the Nitric Oxide Synthases exhibit different profiles during the pancreatic inflammation. The Reactive Nitrogen Species (RSN), produced constantly, are responsible by the regulating the exocrine secretion of the pancreas. At basal levels, RSN protect against pancreatitis by increasing microcirculatory flow, inhibiting the leukocyte infiltration and suppressing the Catepsine, an important mediator to the activation of trypsinogen.

On the other hand, an increase in the Inducible Nitric Oxide Synthase (iNOS) expression followed by an exacerbated Nitric Oxide (NO) production during Acute Pancreatitis could lead to an activation of the inflammatory pathway, inhibition of the cellular respiration, nitrosylation and nitration of proteins, resulting in cell death by necrosis and apoptosis.
In the mammalian cell, the NO is protective against chemistry agents generating of oxidative stress .This anti-oxidant effect could be important to minimize the tissue injury in the ROS-associated process. Once the NO is accumulated in its activated form, its concentration in the tissues is important to the regulation of immunity by modulation of the protein expression and activity.
Pancreatitis is known to cause a marked increase in superoxide production. The key players in the physiology of inflammatory processes are NO and its products. There is great controversy in the literature as to whether the effects of NO are protective or harmful. There is evidence that NO promotes cell death under conditions of oxidative stress, indicating concurrent superoxide production. However, under resting conditions, NO is protective. Recent studies have shown an increase of lipid peroxidation, which indirectly indicates the release of oxidative products, during pancreatitis. This increase was concomitant to enhance the NO production.
It has been shown that, in the presence of high superoxide levels, peroxynitrite formation becomes dependent on NO production. Peroxynitrite can exert its toxic effect through the nitration of macromolecules or as a selective oxidant, contributing to either necrosis or apoptosis. The formation of nitrotyrosine is a consequence of peroxynitrite activity, and increased nitrotyrosine levels have been detected in human diseases associated with oxidative stress. There are various ways in which peroxynitrite-induced impairment of endothelial function might contribute to the pathogenesis of organ failure due to circulatory shock: by exacerbating local vasospasm, increasing local neutrophil adhesion, and increasing neutrophil migration into inflamed tissues; by exacerbating platelet activation and aggregation; or by promoting hypoperfusion of certain parts of various organs. During pancreatitis, it was shown that the hepatocytes around the central vein were apparently the most susceptible to aggression. The superoxide anion and NO both react rapidly to form the toxic reaction product, the peroxynitrite anion. Pharmacological studies in a variety of experimental systems have demonstrated that peroxynitrite is more cytotoxic than is NO or superoxide, as well as being able to induce necrosis and apoptosis. Peroxynitrite (endogenous or exogenous) is a potent trigger of DNA single-strand breakage, whereas NO is not. In turn, DNA single-strand breakage activates the nuclear enzyme poly (adenosine diphosphate-ribose) polymerase.
The concentrations of NO and superoxide determine the formation of peroxynitrite, which was demonstrated after pancreatitis induction. This event is correlated to the increase of liver injury during pancreatitis.

4. Molecular biology of cell death in acute pancreatitis

Acute pancreatitis–associated distant organs injury is mediated by inflammatory cytokines that are produced within tissue resident macrophages. These organs, in turn, participate in the systemic inflammation releasing several inflammatory mediators leading to amplification of the injury of distant organs. Substances released systemically during pancreatitis, such as Nitric Oxide (NO) and free radicals can interfere with mitochondrial respiration and induce apoptosis. The apoptotic cell death may play a considerable role in

affecting mortality and morbidity in severe acute pancreatitis. Apoptosis pathway, by death receptors or the mitochondrial pathway, activates the final caspase to cell death. Death Receptors signaling has been associated with apoptosis in several hepatic diseases such as ethanol-induced liver injury and cholestatic liver disease. Apoptosis related to the severe acute pancreatitis injury is known to be triggered through the mitochondrial pathway.

Cell death has been seen in both apoptotic and necrotic forms, in clinical as well as experimental acute pancreatitis. Current evidence suggests that the amount and the balance between apoptosis and necrosis influence the severity of acute pancreatitis.

There are two apoptotic pathways: the extrinsic pathway is activated by death receptors and is subjected to caspase-8 activation. The mitochondrial or intrinsic pathway is mediated by caspase-9 activation. Both pathways activate the caspase-3, initializing the programmed cell death.

On the other hand, the pancreatitis can activate directly the caspase-9, which forms a complex with Activator Protease Factor-1 and cytochrome c, priming the mitochondrial pathway.

The mitochondria are the determining factor to modulation of cell death during pancreatitis, defining whether the cell death will occur by necrosis or apoptosis. While the necrosis is often observed in severe pancreatitis, apoptosis is more evident in the pancreatitis of medium gravity.

5. Important proteins signaling during acute pancreatitis

The family of protein associated to the membrane fusion machinery includes receptors bind to proteins attachment to N-ethylmaleimide-sensitive fusion proteins and donor vesicles. Some isoforms of this multiprotein complex are present in the pancreatic acinar cells where they mediated the granule-granule fusion as well as the membrane-granule fusion.

The regulation of these proteins is mediated by other protein family called Munc. Recent studies demonstrated molecular interaction of the isoform 18c of Munc and proteins of SNARE family during CCK or carbachol stimulation. This interaction is mediated by modifying in the calcium release. It is important to mention that this basolateral plasma membrane activity is intermediated by protein kinase C family proteins, which are activated by carbachol stimulation.

It was demonstrated that the pancreatitis could induced the Heat Shock Protein (HSP) gene transcription and, in contrast, reduce the protein levels. This modulation is important due the different physiologic roles of each one of the HSP. HSP60, for example, is involved in toll-like receptors activation and necrosis process. On the other hand, the HSP70 is described as a tissue protector in several disease models.

Recently, heat-shock proteins and their cofactors have been revealed associated to apoptotic and necrotic pathways .Heat shock proteins are molecular chaperones that stabilize and refold damaged intercellular proteins, preventing the intracellular protein aggregation and making the cells resistant to stress-induced cell damage.

In the experimental acute pancreatitis, induced by cholecystokinine, cerulean, arginine and taurocholic acid, there is an increase of the Heat Shock Protein (HSP) -70 expression concurrent to a reduction of the HSP60. On the other hand, some authors suggest that HSP60 could reduce the trypsinogen activation, a basic event to the onset of pancreatitis.

During inflammation, neutrophils connect to tissue areas activated. Throughout the neutrophylic invasion, there is the release of enzymes responsible by the tissue digestion, such as metalloproteinases, which in turn could enhance the injury due the release of signaling molecules after the extracellular matrix digestion.
The local injury during pancreatitis induces the production of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). These cytokines are released via portal vein and lymph fluid drainage to the circulation. In turn, it occurs the vascular endothelium activation and the leukocyte migration. In the acute pancreatitis, the increase of pro and anti-inflammatory cytokines was correlated to the reduced human leukocyte antigen-DR levels, which characterizes the immunosuppression. This event could explain the multiple organ failure often related to the pancreatitis.

6. Molecular biology in the treatment of the acute pancreatitis

Currently, the treatment of the acute pancreatitis aims the hemodynamic balance, nutrition, control of the pain and complications. However, the major events in the pancreatitis are: Systemic Inflammatory Response Syndrome, microcirculatory disturb and translocation of bacteria.
Several experimental studies demonstrate the efficiency of Nitric Oxide, block of endothelial receptors, antagonists of the PAF-receptors, antibodies against Intercellular Adhesion Molecules. These treatments improved the microcirculatory flow during acute pancreatitis.

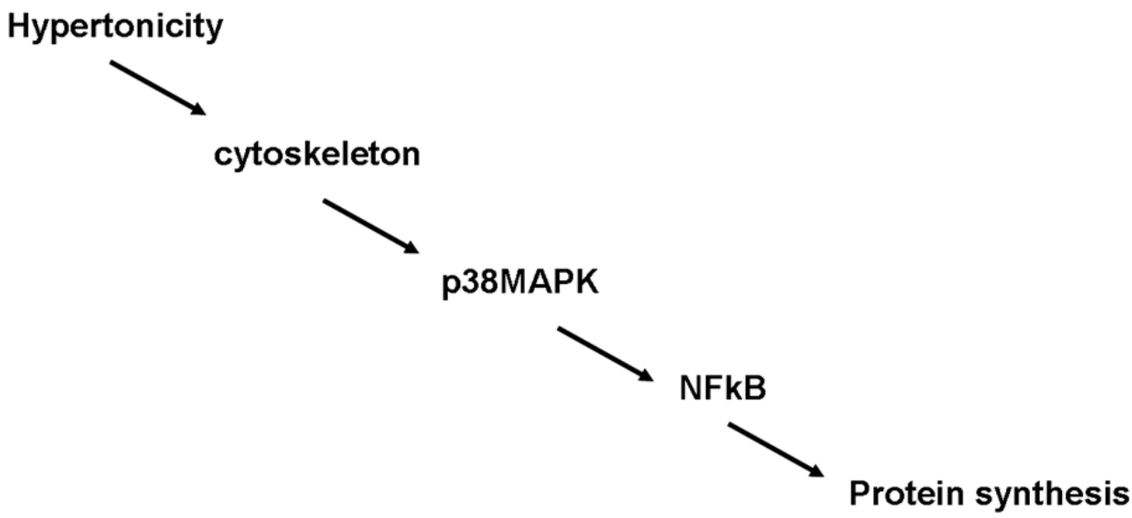

Fig. 2. Molecular effects of the hypertonic solution. The water loss could modify the cytoskeleton structure leading to activation of a protein cascade triggering specific gene transcription.

Fluid resuscitation is a necessary therapeutic intervention in severe pancreatitis. Patients with pancreatitis present volume extravasation to the peritoneum and retroperitoneum, and some have hemodynamic inestability. However, the infusion of large volumes can induce pulmonary interstitial edema and can increase intra-abdominal pressure. Fluid accumulation in the lungs exacerbates respiratory failure and can make mechanical

ventilation necessary. Increased abdominal pressure reduces the venous return to the heart, thereby decreasing cardiac output, as well as reducing perfusion of the kidney and gut, all of which can provoke organ damage.

In experimental animal models of pancreatitis, hypertonic saline has been shown to alter circulating plasma volume, reduce trypsinogen levels, prevent acinar necrosis, reduce inflammatory cytokine levels, and avert pancreatic infection, thereby minimizing injury, limiting the local and end-organ and reducing mortality. It has recently been demonstrated that administration of hypertonic saline in a rat model of acute pancreatitis reduces systemic inflammation rather than protecting local (pancreatic) tissue. Hypertonic saline modulates pancreatitis-related injury to the lungs and liver. This effect was attributed to the modulated expression and activity of various proteins, such as metalloproteinase, collagen, and members of the HSP family. Also hypertonicity could modify the gene transcription, expression and function of several proteins, activation of kinases (figure 2), cell adhesion and the ROS and cytokines release. This modifying may be due the alteration in the cytoskeleton because the cell edema diminishing.

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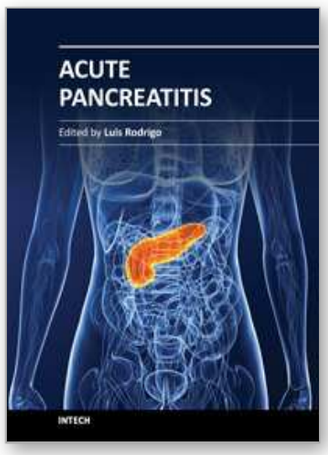

Acute Pancreatitis

Edited by Prof. Luis Rodrigo

ISBN 978-953-307-984-4
Hard cover, 300 pages
Publisher InTech
Published online 18, January, 2012
Published in print edition January, 2012

Acute Pancreatitis (AP) in approximately 80% of cases, occurs as a secondary complication related to gallstone disease and alcohol misuse. However there are several other different causes that produce it such as metabolism, genetics, autoimmunity, post-ERCP, and trauma for example... This disease is commonly associated with the sudden onset of upper abdominal pain that is usually severe enough to warrant the patient seeking urgent medical attention. Overall, 10-25% of AP episodes are classified as severe. This leads to an associated mortality rate of 7-30% that has not changed in recent years. Treatment is conservative and generally performed by experienced teams often in ICUs. Although most cases of acute pancreatitis are uncomplicated and resolve spontaneously, the presence of complications has a significant prognostic importance. Necrosis, hemorrhage, and infection convey up to 25%, 50%, and 80% mortality, respectively. Other complications such as pseudocyst formation, pseudo-aneurysm formation, or venous thrombosis, increase morbidity and mortality to a lesser degree. The presence of pancreatic infection must be avoided.

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