Ascites fluid in severe acute pancreatitis : from pathophysiology to therapy

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Abstract

Several pathophysiological mechanisms are involved in the development of the inflammatory necrotizing process that takes place in the retroperitoneal area during the early phase of acute pancreatitis. They include premature intraglandular activation of pancreatic proenzymes (zymogens) and in particular trypsin, early microcirculatory impairment with subsequent ischaemia/reperfusion and overstimulation of immune effector cells. Although intra-acinar or interstitial activation of trypsinogen is most probably the trigger of acute pancreatitis, in recent years much emphasis has been put on the role of leukocytes. Based on numerous experimental and human data several pro-inflammatory mediators including cytokines, arachidonic acid derivatives, activated oxygen species and proteases are released locally by overactivated neutrophils and monocytes/macrophages among other cells. They are now believed to play a central role in the development of pancreatic necrosis and, once they gain access to the systemic circulation, in the emergence of early multisystem organ failure. However the sequential and relative contribution of each of these 3 pathophysiological mechanisms remain controversial and the precise identification of the mediators incriminated in local and remote tissue injury is still awaited.

Severe acute pancreatitis still carries a mortality of 20% to 30%. With advances in intensive care management 80% of the deaths occur somewhat late in the attack due to infected pancreatic necrosis. Nevertheless early remote organ failures still remain a lifethreatening condition for most of these patients. A peritoneal exudate rich in activated lipolytic and proteolytic enzymes, vasoactive substances and several other pro-inflammatory mediators collect in over 60% of the patients with severe acute pancreatitis. On the basis of favourable animal experiments early percutaneous or surgical peritoneal lavage with or without the addition of antiproteases has been carried out in human acute pancreatitis. The rationale behind this procedure was the washout of potential toxic mediators from the peritoneal cavity before they gain access to the systemic circulation. Contrary to animal and uncontrolled human data no prospective randomized study could ever demonstrated a significant effect of peritoneal lavage neither in the prevention and control of remote organ failures or in early mortality and ultimate survival after severe acute pancreatitis in humans. Differences between experimentally-induced pancreatitis, difference in the timing of the initiation of lavage and a type II error in controlled human studies may account for the discrepancy in the outcome between these studies. Anyway, this disparity should raise the question as whether the peritoneal cavity acts simply as a reservoir or as a route of transfer of toxic mediators to the systemic circulation. Although data are scarce, conflicting and limited to animal experiments and to a few molecules, peripancreatic veins and lymphatics seem to be the major routes of transfer whereas transperitoneal absorption is trivial.

Nevertheless early peritoneal aspiration of ascitic fluid in acute pancreatitis and measurement of trypsinogen activation peptides may be used as a means of severity assessment and identification of pancreatic necrosis. This implies that even if not taking part actively in the emergence of remote organ failures ascitic fluid may reflect the peripancreatic necrotizing process. So careful comparative analysis of peritoneal exudate, plasma and lymph with regards to putative mediators of local and remote injury may provide essential pathophysiological clues. At the time of trials of antimediator therapy early in the attack this kind of insight is essential. (Acta gastroenterol. belg., **2000**, 63, **264-268**).

Key words : acute pancreatitis, ascites, peritoneal lavage, peritoneal dialysis.

Introduction

Severe acute pancreatitis (SAP) defined as a fatal attack or the emergence of at least one local or remote complication develop in 10 to 20% of acute pancreatitis. Ultrastructurally SAP is characterized by an intense locoregional inflammatory process with ensuing patchy or coalescent necrotic areas which involve a variable portion of the gland and at times surrounding retroperitoneal tissues (1). Systemic complications and in particular cardiorespiratory failure occur typically in the early phase of the attack and may culminate in multisystem organ dysfunction syndrome (MODS). Locoregional events, particularly infected pancreatic necrosis, usually complicate the course later when necrotic areas have constituted. Although remote organ failures still carry a significant morbidity (2), nowadays advances in intensive care medicine enable most patients to survive early MODS so that 80% of the mortality of SAP should be attributed to pancreatic infection (1,3,4). Though not definitely proven in humans bacterial translocation from the gut should play a central role in the pathogenesis of infected necrosis (5,6). Prevalence of infection ranges from 40 to 70% in necrotizing pancreatitis depending on the volume of necrotic areas and the length of exposure (3,4,7-9). Thus prognosis in SAP is influenced by the emergence of early MODS, the development and the extent of locoregional necrosis and the bacterial contamination of these necrotic areas (3). Sterile necrosis carries a mortality of 10% vs 20-40% in case of pancreatic infection (3,7,10,11).

The inflammatory necrotizing process in the retroperitoneal space generates a peritoneal exudate that collect in over 60% of the patients with SAP. This fluid is rich in activated lipolytic and proteolytic enzymes, vasoactive substances and several other pro-inflammatory mediators. Herein potential pathophysiologic, prognostic and therapeutic implications of pancreatic ascites are briefly reviewed.

Pathophysiology of locoregional necrosis and remote organ dysfunctions : role of pancreatic ascites

Despite the natural safeguards of the pancreas against autodigestion it is now widely accepted that the *prema-*

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ture activation of trypsin triggers the locoregional necrotizing inflammatory process as soon as these safeguards are exhausted (12). The earliest pathophysiological events take place inside the acinar cell and they all alter the normal exocrine secretory function (13). Whatever the aetiological factor in humans or the experimental model in animals abnormal elevation of intracytoplasmic calcium modifies the secretory polarity of the cell towards the baso-lateral membrane and the insterstitium of the gland (14,15) and favours the auto-proteolysis of trypsinogen as well as the colocalization of the proenzyme with lysosomal hydrolases and in particular cathepsin (crinophagy) (13,16,17). The precise location (interstitial or intra-acinar) and the predominant mechanism (autoactivation or crinophagy) of this premature conversion of trypsinogen into trypsin remain undetermined (18,19). Nevertheless, once trypsin is generated in excess of local (pancreatic secretory trypsin inhibitor) and regional (alpha1 proteinase inhibitor – alpha 2 macroglobulin) inhibitors this protease in turn activates both the other pancreatic zymogens and several cascade systems of proteases including the coagulation, fibrinolytic, kallikrein-kinin and complement systems (20).

Severe *impairment in the pancreatic microcirculation* is an early and constant feature which is ascribed to the interaction of premature activated enzymes (trypsin, elastase, C5a) and secondary oxydative stress with endothelial cells (21,22). This microcirculatory failure extends the glandular damage initiated by the premature cascade activation of zymogens. Among others, ischaemia disrupts the normal intracellular segregation between zymogens and lysosomal hydrolases and once reperfusion ensues activated oxygen species inactivate antiproteases (23).

The cellular damage that is initiated by these enzymatic activations and microcirculatory disturbances is responsible for the *secondary recruitment and activation of immune effector cells*, endothelial cells and fibroblasts in the pancreatic area (24). Both C5a and leukotrienes are probably the initial chemoattractants while uncontrolled transcription and release of pro-inflammatory cytokines (TNF-α,IL-1β) by acinar cells and macrophages soon enhance leukocyte invasion of the gland and leads to overactivation of endothelial and immune effector cells (25-27). These cells produce potent inflammatory secondary mediators including pro- and anti-inflammatory cytokines, chemokines, arachidonic acid metabolites, proteases and activated oxygen species. This initial normal response to cellular damage is a local inflammatory process that mobilize the immune response and promotes healing. If, however, the control mechanisms that localize this response are ineffective or overwhelmed by the intensity of initial glandular damage the inflammatory response becomes amplified, further fuels the pancreatic necrotizing process locally and may spread systematically, unleashing a cascade of mediators that exert profound deleterious effects on organ systems throughout the body (28). Uncontrolled release of pro-inflammatory cytokines (TNF-α,IL-1β) and chemokines (IL-8, PAF) leads to activation of immune effector cells at distance of the pancreas and to the multifocal synthesis of common toxic mediators (27).

This cascade of events responsible for the locoregional necrosis and remote organ failures in patients with SAP has been in recent years the focus of intense efforts to map the process in order to identify possible strategies for therapeutic interventions and in particular agents able to interfere with key mediators in the process (29). Unfortunatelly due to the lack of an animal model that reproduces human disease as well as the multiple redundancies and interactions between each set of mediators it has not been possible to delineate precisely the relative contribution of the 3 pathophysiological mechanisms in the progression of locoregional necrosis and the emergence of distant organ dysfunctions. Similarly the accurate identification of key mediators incriminated in local and/or remote tissue injury and which are not necessarilly similar, has not been achieved neither their mode of access at distant sites.

With this perpective in mind ascitic fluid that collects within the peritoneal cavity during the early days of SAP as a result from the contiguous extension of the locoregional necrotizing process may be considered either as a *route of transfer of toxic mediators* to the systemic circulation or as *a unique compartment that mirrors what happens around the gland.* Thus careful analysis of the peritoneal exudate with regards to putative mediators of local and remote tissue injury and physiological defence mechanisms may provide important pathophysiological clues. At the time of trials of antimediator therapy early in the attack this kind of insight is essential.

Conflicting experimental data have been reported on the role of ascitic fluid in the *transfer of toxic mediators* to the systemic circulation. From experimental pancreatitis induced in dogs and in pigs in which the gland was isolated from the peritoneal cavity Egdahl aswell as Waterman and Walsky concluded that the dominant route of enzyme transfer was via the thoracic duct lymph due to transperitoneal absorption of enzyme-rich fluid into peritoneal lymphatics (30,31). Popper and Necheles as well as Howard et al noted that the serum amylase level did not rise immediately after induction of pancreatitis when the portal vein was obstructed (32,33). This led to the suggestion that direct venous absorption of enzymes from the inflammed gland was the principal determinant of transfer. Later on similar conclusions were reached by Lange et al in experiments carried out in the rat (34). Enzyme absorption by the thoracic duct was severely limited in this species by a rapid cessation of lymph flow after induction so that portal pathway of enzymes transfer was dominant and transperitoneal absorption from pancreatic ascites was mainly hematogenous. Lastly Mayer et al investigated in dogs the relative contribution of ascitic fluid and thoracic duct lymph as route of transfer of pancreatic enzymes from

the inflammed gland to the blood (35). By collecting the exudate from the gland in some animals and cannulating the thoracic duct in others they showed that the initial rise of serum enzymes was because of the direct transfer into pancreatic veins and thereafter transfer of enzymes via retroperitoneal lymphatics was dominant. The majority of enzymes released inappropriately from the gland accumulated within the ascitic fluid but transperitoneal absorption was negligible. Interspecies and interexperiment differences may account for much of the discrepancy in findings between these studies. Although no specific data exist on pathways of mediator transfer in human pancreatitis the negligible influence of peritoneal lavage upon the plasma concentrations of pancreatic enzymes lends support to Mayer's hypothesis (36). However an increased peritoneal blood flow and a secondary increased peritoneal permeability to relatively small molecules have been demonstrated in experimental pancreatitis (37,38). These alterations are thought to be a consequence of vasoactive substances in the peritoneal exudate and contribute to the accumulation of fluid in the peritoneal cavity. Thus even if little amylase or lipase leaves the peritoneal sump of fluid the same fate may not be shared by other proteases, bioactive peptides and relatively small molecules which may pass more readily into the plasma, either directly into peritoneal capillaries or into peritoneal lymphatics and may contribute to the systemic response to the attack. This has prompted interest in the analysis of pancreatic ascites with respect to several classes of mediators.

High levels of *trypsin, chymotrypsin and pancreatic elastase* in complex with α1-proteinase inhibitor and α2 macroglobulin are found in the ascitic fluid of patients with SAP (39). The presence of complexes between activated proteases and α2 macroglobulin is explained in severe attacks by an intense periglandular release of trypsin and other activated pancreatic enzymes with subsequent complex formation aswell as by a relative deficiency in the clearance of complexes by the reticuloendothelial system. Marked protease inhibitory consumption and a local protease-antiprotease imbalance were demonstrated as α2 macroglobulin is a high-molecular weight intravascular protein with limited extravascular access and low-molecular-weight inhibitors, like α1-proteinase inhibitor, act as carrier proteins for activated proteases and in particular trypsin and thus offer limited inhibitory capacity. In addition oxidation of the active site of α1-proteinase inhibitor by activated oxygen species further reduces its binding capacity. The presence of free active trypsin could be demonstrated by gel filtration chromatography (personal unpublished observation). So there is evidence of proteolytic enzyme activity within the peritoneal fluid (40). This fluid increased vascular permeability and induced hypotension and death when injected intravenously or intraperitonally into healthy animals. Local activation of the complement, kinin, fibrinolytic and coagulation systems is found as soon as α2 macroglobulin concentration falls below 30% of normal and in spite of 90% free and reactive α1-proteinase inhibitor (39). The extent of these activations and prior consumption of α2-macroglobulin is closely correlated to the severity of pancreatitis. Similarly the measurement of trypsinogen activation peptides in the peritoneal fluid, which are released from the conversion of trypsinogen into trypsin, compared with contrast enhanced computed tomography in the detection of pancreatic necrosis and early assessment of severity (41). Pancreatic enzyme concentration, changes in protease-antiprotease balance and secondary continuing activations including those of zymogens are predominant in the peritoneal fluid when compared with blood, which supports the concept that enzymes released inappropriately from the gland collect continuously within the peritoneal cavity (35). This indicates also the close vicinity of this compartment with the necrotizing process and the potentiel relevance of the biochemical analysis of this fluid if not to the pathophysiology of remote organ damage at least to the one of retroperitoneal injury. In blood antiproteases prevent the existence of free proteolytic enzymes.

There are relatively few data on mediators released in the peritoneal exudate by *overactivated immune effector cells*. Similarly to pancreatic proteases ascitic fluid on admission for SAP contains high concentrations of leukocyte elastase and neutrophil protease 4, both largely in excess of the high initial levels found in plasma (42). This points to a massive release of leukocyte proteases within the confined pancreatic space. In humans phospholipase A2 activities and concentrations are increased in serum and other body fluids (43). Levels of this enzyme which hydrolyses phospholipids of cell membranes into cytotoxic lysophospholipids and free fatty acids including arachidonic acid are maximal in the peritoneal fluid. Metabolites of the arachidonic acid cascade and in particular the lipooxygenase pathway are potent vasoactive mediators and contribute to the inflammatory reaction by increasing vascular permeability, by producing ischaemia and by attracting neutrophils. In experimental pancreatitis high concentrations of leukotrienes C4 and D4 were found in the ascitic fluid (44). In the rat pancreatic ascites has been shown to contain soluble unidentified factors that can upregulate both the expression of adhesion molecules in human vascular endothelial cells, at least in part through the activation of nuclear factor κ B aswell as the IL-1 and TNF genes within the lung of healthy animals infused with this fluid (45,46). Thus this exudate may be important stimulus fo endothelial activation and leukocyte recruitment into distant organs during SAP.

Diagnostic and prognostic yields of pancreatic ascites

A peritoneal tap can provide at times useful corroborative evidence of acute pancreatitis, especially if sterile ascitic fluid with a high amylase concentration is

aspirated. Reliance upon clinical features and hyperamylasemia alone may result in an incorrect diagnosis. In a prospective study of the prognostic value of peritoneal lavage in patients with acute pancreatitis the procedure enabled an erroneous clinical diagnosis to be corrected in 7 out of 373 patients (1,9%) and confirmed a tentative clinical diagnosis in 2 other patients (47).

Early assessment of severity and detection of necrotizing pancreatitis are of outstanding importance in order to monitor the patient closely though admission to an intensive care area, to anticipate early and late complications and to consider aggressive treatment directed at necrosis and its local and systemic consequences. As "prune juice" colored ascitic fluid is present in the peritoneal cavity of more than 50% of the patients with necrotizing pancreatitis who undergo early surgery, abdominal paracentesis may provide useful prognostic information. By considering the volume and the colour of free ascitic fluid or the colour of lavage fluid using a standard colour chart, 90% of the patients who died and 72% of the severe attacks could be identified within 5 h of admission (47). However unlike biochemical markers (C-reactive protein, granulocyte elastase) and some grading systems of multiple clinical and laboratory criteria (APACHE II-score) repetitive assessment is not feasible. Since the prognostic information is based on early data, monitoring of the course of the attack is impossible and accurate prediction of late complications is poor. Moreover this procedure is flawed by a rather low sensitivity and by a 0.8% risk of visceral perforation. Thus in spite of its speed and availability peritoneal aspiration has never been accepted widely into clinical practice.

Despite its prognostic performance measurement of trypsinogen activation peptides in pancreatic ascites has not gained wide acceptance because of its lack of availability (41).

Therapeutic value of peritoneal lavage in SAP

Several controlled animal studies showed a positive effect of peritoneal lavage on the survival curve of experimentally-induced pancreatitis. The addition of a low molecular weight protease inhibitor to the lavage fluid and the intraperitoneal replenishment of natural protease binding proteins (α2 macroglobulin) with fresh frozen plasma further reduced mortality (48,49). Importantly none of these animal studies employed a protocol that delayed therapeutic lavage > 4-6 hours after induction of the attack.

In man prospective uncontrolled studies showed a striking immediate improvement accompanied by relief of pain and reversal of the early phase cardiorespiratory disturbances, especially in the subgroup of alcohol-related pancreatitis (50,51). Contrary to experimental trials the addition of antiproteases to the lavage fluid did not convey additional benefit (52). In all these studies the potential benefits of peritoneal lavage were ascribed to the removal of toxic substances from the peritoneal cavity before they gain access to the systemic circulation. It seems unlikely that enough lavage fluid gets access to the lesser sac where the concentration of toxic agents must be greatest. So early but not overall mortality was reduced since lavage did not alter the progression of pancreatic tissue injury. In particular except in a single uncontrolled trial of long peritoneal lavage this procedure did not influence the incidence of infected necrosis.

Unfortunately the two human prospective randomized studies of peritoneal lavage did not report statistical significant improvement in either early or ultimate survival nor in the prevention and control of remote organ failures (35,53). Discrepancy in the results between experimental and controlled human studies should be ascribed to several factors including differences between animal models and human disease and lack of statistical power for human investigations to have confidence in the negative outcome. Fundamentelly the delay in delivering treatment in humans should have crucially undermined the effectiveness of lavage. Given the complexity and interrelations between the pathophysiological mechanisms underlying pancreatic and remote tissue injury aswell as the multiple pathways of transfer of toxic mediators it is unwise to expect major benefit from a single procedure that is instituted days after onset of disease and adresses at most only one of the route of transfer or at worst a simple reservoir of enzymes. It is the clinical reality that patients with SAP are not available for treatment until the triggering mechanism is long past. At that time the washout of a single compartment even coupled with local antiprotease therapy is too little and too late. In the most severe cases further tissue injury might be prevented by external channeling of the flow of activated enzymes by surgically-inserted drains in the retroperitoneum (54). Nevertheless given the impressive clinical improvement associated with early peritoneal lavage in some patients with SAP this procedure might still be part of a conservative therapeutic strategy despite the conflicting results of controlled studies.

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