The Role of Calcium and Pro-Inflammatory Mediators in Acute Pancreatitis

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Abstract

Acute pancreatitis is a common condition that is potentially fatal in severe cases. Pancreatic acinar cell injury in acute pancreatitis results in inflammation. This causes a variety of systemic complications including organ damage, fat necrosis and vascular damage. Many aetiological factors responsible for causing acute pancreatitis have been identified. But the exact mechanisms through which they cause acute pancreatitis remain unclear. With increasing incidence rates of acute pancreatitis over the years and the lack of a definitive treatment, it is important that research continues in order to find successful treatment. This review analyses available literature in order to find the role of calcium and pro-inflammatory mediators in acute pancreatitis.

Introduction

The pancreas is a gland located in the upper, posterior abdomen. It extends retroperitoneally from the second part of the duodenum towards the spleen (1). The pancreas is made up of small clusters of glandular epithelial cells which contribute towards its exocrine and endocrine function.

The exocrine part of the pancreas consists of clusters of acini which are drained by ductules (2,3). The acinar cells are typical exocrine glandular cells that synthesise and secrete enzymes which eventually flow through the pancreatic duct into the duodenum (4). These cells are pyramidal epithelial cells that are arranged in rows. The apaxes of the acinar cells join to form the lumen of the acinus. In addition to secreting enzymes, that pancreas also secretes pancreatic juice which contains water, ions and a variety of proteins. The sodium bicarbonate in the pancreatic juice is slightly alkaline (pH 7.1-8.2) and buffers the acidic gastric juice that enters the small intestine along with the chime (5). This provides an optimum pH for the activity of the pancreatic enzymes. Pancreatic enzymes are necessary for the digestion and absorption of fats, carbohydrates and proteins.

Zymogen granules found in acinar cells release the enzymes they contain by exocytosis into the lumen. Enzymes secreted in the active form by the pancreas include lipase, amylase, deoxyribonuclease, and ribonuclease. Zymogens (proenzymes) include trypsinogen, chymotrypsinogen, proelastase, procarboxypeptidase and phospholipase A₂. In the small intestine trypsinogen is converted into its active form trypsin by the intestinal brush-border protease enterokinase. Trypsin then converts the remaining zymogens into their active forms (6). Trypsin can also activate trypsinogen, producing the potential for a autocatalytic chain reaction (7).

Regulation of Pancreatic Secretion

Pancreatic secretion is regulated primarily by two different hormones: secretin and cholecystokinin (CCK).

Secretin is secreted by the S cells found in the duodenum and jejunum, in response to gastric acid in the duodenum. Pancreatic duct cells secrete watery fluid containing bicarbonate ions and enzymes with low activity upon stimulation by secretin (7).

CCK is secreted by I-cells in the duodenal mucosa as a result of amino acids, peptides and fatty acids entering the duodenum. CCK causes the zymogen granules present in the pancreatic acinar cells to release their enzymes. The pancreas also secretes a small amount of pancreatic juice which is also rich in enzymes. The release of enterokinase is also increased as a result of CCK (7).

Furthermore, acetylcholine (Ach) released by the vagus nerve can also cause exocytosis ofzymogens by the zymogen granules in the acinar cells (7,8).

Pancreatitis

Pancreatitis can be classified as either chronic or acute. Acute pancreatitis is defined as an acute inflammatory process of the pancreas that can involve peripancreatic tissues or remote organ systems, or both (9). Acute pancreatitis can be further classified into mild and severe forms. Mild acute pancreatitis is associated with minimal organ dysfunction whilst severe acute pancreatitis is associated with pancreatic necrosis which may lead to organ failure (10).

Chronic pancreatitis is defined as chronic inflammation, irreversible structural changes, and permanent impairment of exocrine and endocrine pancreatic function (11).
The overall mortality rates of acute pancreatitis is 5-10% and up to one fifth of patients who develop serious acute pancreatitis die (12,13). This poses a serious problem to public health given that the incidence rates have also been rising over the last few decades (14). Around 80% of cases of acute pancreatitis can be attributed to gallstones and excess alcohol consumption (15).

Clinically acute pancreatitis presents as acute upper abdominal pain, nausea, vomiting and fever. A raised serum amylase differentiates pancreatitis from other conditions that also cause upper abdominal pain (7). Currently there is no specific treatment for pancreatitis and given the increasing incidence rates, it is imperative that we continue research on the mechanisms of pancreatitis in order to find a therapeutic treatment as they are not fully understood yet (15).

Acute pancreatitis is essentially autodigestion of the pancreas. There are protective mechanisms that attempt to prevent autodigestion from occurring. Pancreatic secretory trypsin inhibitor (PSTI) which is also known as serine protease inhibitor, kazal type 1 (or SPINK1) inhibits approximately 20% of trypsin activity. If this fails due to the SPINK1 inhibitory activity being overwhelmed or ineffective, then trypsin inactivation can occur through trypsin autolysis (7).

Methods and Results

Relevant books on intracellular signalling and pancreatitis were obtained through the Harold Cohen Library. Online virtual seminars were accessed via the Henry Stewart Talks website but unfortunately they only offered sample extracts. Other websites accessed included the World Health Organization and the National Institute for Clinical Excellence (NICE). However, the majority of them lacked useful information on the pancreas and pancreatitis.

A wide range of online databases were used to conduct online searches. These included Medline via the Ovid search engine, Pubmed, Google Scholar, ScienceDirect and Wiley InterScience. Many of these were accessed through the University of Liverpool in addition to the universities online e-books and e-journals.

MeSH (medical subject heading) terms used on Medline database via the Ovid search engine included acute pancreatitis, calcium and inflammatory mediators. To cut down the number of results, limits were applied on the searches that included language (English), species (human) and type of article (Clinical Trial, Meta-Analysis, Randomised Control Trial, Review). Articles that focused too much on chronic pancreatitis or that were deemed irrelevant were disregarded for the purpose of this review. The results of the searches are shown in table 1.

Discussion

Acute pancreatitis stems from a result of a complex cascade of events which begin in the pancreatic acinar cells. The exact mechanisms are not yet fully understood. However, there is a common theory which is widely accepted. This theory suggests that acute pancreatitis is a result of injury or disruption of the pancreatic acini, which allows leakage of pancreatic enzymes (trypsin, chymotrypsin and elastase) into pancreatic tissue (16). These enzymes are prematurely activated resulting in autodigestion of the pancreas and therefore acute pancreatitis. The central event surrounding acute pancreatitis is the intra-acinar activation of trypsin from trypsinogen (17). Trypsin then overwhelms the defense mechanisms and activates other zymogens. One study by Kukor et al (18) using a purified enzyme showed that calcium is important for the activation of anionic and cationic trypsinogen (18). An acidic environment is also reported to be important for trypsinogen activation (19).

Pancreatic lipase is released due to pancreatic acinar cell damage. This enzyme which is released in its active form breaks down surrounding adipose tissue resulting in fat necrosis. Once Trypsin has been activated, it activates the proenzymes of chymotrypsin, elastase, and phospholipase A. The activation of chymotrypsin causes oedema and vascular damage whilst activation of elastase from proelastase leads to the digestion of elastin in blood vessels. This causes vascular injury and haemorrhage. Activation of the kallikrein-kinin system by trypsin causes the release of bradykinin and kallidin which results in vasodilation, increased vascular permeability and inflammation (7,20).

The Role of Calcium

Calcium is an intracellular second messenger that has a variety of effects in the body. These include muscle contraction, control of cell growth, activation of platelets, control of secretion and apoptosis (15).

The cytosolic calcium ion concentration ([Ca$^{2+}$]) of the resting pancreatic acinar cell is in the order of 10$^{-7}$ M. In the extracellular fluid the concentration of calcium is 10$^{-3}$ M whilst in the endoplasmic reticulum it is 10$^{-4}$ M (21). This creates a concentration gradient which
favours entry of calcium into the cytosol. Actions of a plasma membrane Mg²⁺-dependent Ca²⁺-ATPase (22) and an endoplasmic reticulum Ca²⁺-ATPase maintain this concentration gradient (23). Maintenance of a low [Ca²⁺] is important as it enables small local increases in the concentration of cytosol calcium to be utilised as a signal to control intracellular events (24). Furthermore high levels of [Ca²⁺], are toxic to many types of cells (24,15).

The apical calcium signalling complex consists of four separate calcium stores which are the endoplasmic reticulum (ER), the zymogen granules (ZG), the endosomes and the lysosomes (25). The basolateral membrane is the principal site for hormone (CCK) and neurotransmitter (Ach) interaction with their specific receptors (26). The binding of Ach to muscarinic receptors on the basolateral membrane activates phospholipase C (PLC) via the G-protein pathway. Subsequently inositol 1,4,5-trisphosphate (IP₃) is generated. CCK also activates PLC, but only at high, unphysiological concentrations. PLC activation has not been shown at normal physiological levels of CCK (27).

The inner surface of the basolateral membrane is where the intracellular Ca²⁺ releasing messengers are most likely to be generated. IP₃ is an intracellular Ca²⁺-releasing messenger that is thought to be very mobile in the intracellular fluid (28). Furthermore, two other intracellular Ca²⁺ releasing messengers, cyclic ADP ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP) are thought to be very mobile in intracellular fluid due to their small water soluble structure (29). Therefore, due to their mobility in intracellular fluid, IP₃, along with cADPR and NAADP can be regarded as long-range intracellular messengers. Conversely, the fourth Ca²⁺ releasing messenger, Ca²⁺, is regarded as a short-range intracellular messenger due to its low mobility as a result of binding to fixed buffers (28). All four of these messengers act upon Ca²⁺ stores in the apical pole of the pancreatic acinar cells. The calcium stores mentioned above contain IP₃ receptors (IP₃R) and ryanodine receptors (RyR). The activation of the IP₃R by IP₃ or RyR by cADPR or NAADP results in calcium release from internal stores (28).

Mualem reported that after pancreatic acinar cells had been stimulated by Ach or CCK, a rise in the concentration of free calcium ions had been observed in the cytosol (30). Furthermore, Petersen et al (31) reported that CCK hyperstimulation can cause sustained cytosolic Ca²⁺ elevation. This is due to emptying of the intracellular Ca²⁺ stores and subsequent opening of store-operated Ca²⁺ channels in the plasma membrane (31,32).

One study by Krüger et al (33) involved inducing pancreatitis in rodents via a secretagogue. They found that trypsinogen activation began in the apical region of the pancreatic acinar cell and then spread throughout the cytosol (33). Interestingly, they also found that this did not propagate through gap junctions to neighbouring acinar cells (33). This could be thought of as a protective mechanism that limits the amount of autodigestion in the pancreas once premature enzyme activation has begun. Krüger et al (33) also found that trypsinogen activation could only be detected in regions of the acinar cell that had a prolonged Ca²⁺ release of more than 100 seconds (33). They found that premature activation of proteases was greatly dependent on the duration of the Ca²⁺ signal because neither a brief Ca²⁺ peak or oscillation nor a rapid or prolonged increase in intracellularCa²⁺ concentrations in regions other than the apical compartment was followed by subsequent protease activation (33). Further experiments that were carried out involved reducing the intracellular pool of Ca²⁺ and removing extracellular Ca²⁺. This was followed by supramaximum hormone stimulation of the acinar cell. They found that intracellular protease activation, which is a central step in acute pancreatitis, was greatly reduced or abolished (33).

The Role of Pro-Inflammatory Mediators

Inflammatory mediators appear to play a vital role in the pathogenesis of pancreatitis due to the inflammatory response (16).

Nuclear factor-κB (NF-κB) is an important transcription factor that induces the synthesis and release of various cytokines and chemokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-1?. Once activated by various factors (34), NF-κB is released by its inhibitor and it translocates into the nucleus (34). NF-κB then modulates the transcription of various cytokines by activating gene transcription. Reduction in the severity of pancreatitis is shown by inhibition of NF-κB activation in the caerulein hyperstimulation model of pancreatitis (34). This shows that NF-κB has a central role in the inflammatory response of pancreatitis.

During the onset and progress of acute pancreatitis, levels of TNF-α and IL-1? are elevated. Denham et al (35) showed that when acute pancreatitis had been induced on knockout mice who lacked receptors for TNF-α and IL-1?, they had higher survival rates than wild-type mice who lacked the receptors (35). One study showed that pancreatic acinar cells released
increased amounts of TNF-α 30 minutes after caerulein hyperstimulation induced pancreatitis (36). In addition to TNF-α and IL-1β, IL-6 is also a pro-inflammatory cytokine whose levels rise 100 fold in hormone induced pancreatitis (37). The degree of IL-6 induced inflammation correlates with the severity of pancreatitis (38).

The levels of PAF (platelet-activating factor), which reportedly can be synthesised in pancreatic acinar cells, also rise during the course of pancreatitis (39). Substance P, a neuropeptide released from afferent nerve endings in many tissues, plays a central role in the severity of pancreatitis and in acute lung injury (34). Bhatia et al (40) showed that substance P may be upregulated and they also found that mice deficient in the NK1 receptors were protected against pancreatitis and pancreatitis-associated lung injury (40).

Further Study

Further research is still needed to understand the exact mechanism of premature zymogen activation. Hopefully once this is successful, therapeutic treatment may be developed for pancreatitis. Furthermore, the exact role of pro-inflammatory mediators such as substance P and the activation of cytokines in pancreatitis are also not fully understood. These areas could be targeted for therapeutic treatment to help reduce the severity of pancreatitis.

Conclusion

Results from many studies suggest that there is a direct link between pro-inflammatory mediators and the severity of acute pancreatitis. Figure 8 summarises the factors that determine the severity of acute pancreatitis. A concrete link between calcium and pancreatitis has not yet been found but evidence suggests that calcium does play a central role. The mechanism of premature trypsinogen activation is not fully understood yet. At present, we do not understand how a sustained elevated [Ca^{2+}], in combination with Ca^{2+} depletion of the ER as well as the secretory granules, prematurely triggers trypsinogen activation that ultimately leads to acute pancreatitis (41). However, data does suggest that sustained elevations in [Ca^{2+}] is crucial in premature trypsinogen activation (33,16).

References

19. Waterford SD, Kolodecik TR, Thrower EC, Gorelick FS. Vacuolar ATPase regulates zymogen
Illustrations

Illustration 1

Table 1

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