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Review Article

Role of Endogenous Peptides and Enzymes in the Pathogenesis of Acute Pancreatitis: A Review

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Abstract

Acute pancreatitis is an inflammatory disease with the clinical manifestation of acute abdominal pain. Several factors are involved in the pathogenesis of acute pancreatitis. The exact mechanism(s) by which diverse etiological factors induce an attack are still unclear. However, one of the proposed mechanisms for induction of acute pancreatitis is auto-digestion of pancreatic tissues by unwanted activation of pancreatic digestive enzymes. The main objective of this review is to describe the pathogenesis mechanism of acute pancreatitis that are related to various inflammatory and pro-inflammatory mediators.

Keywords: *Acute pancreatitis, Pancreatic injury, Cytokines, Chemokines, Inflammation*

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INTRODUCTION

Acute pancreatitis is an inflammatory disease characterized by varying degrees of endogenous pancreatic injury ranging from a mild oedematous and interstitial process to one which is associated with extensive pancreatic and peripancreatic necrosis. Complications of acute pancreatitis include the development of pancreatic pseudocysts, pancreatic ascites, and vascular changes such as splenic vein thrombosis and development of arterial false aneurysms in the area of the pancreas [1]. The most severe form of acute pancreatitis is severe haemorrhagic and necrotizing pancreatitis, which has been reported to result in mortality rates ranging from 30 to 50 % through liver and lung injury [2]. In particular, liver injury is a clinical prognostic indicator and is incorporated into the various scoring systems used to predict the severity of acute pancreatitis [2,3].

There are several factors influencing the pathogenesis of acute pancreatitis like premature activation of trypsinogen, chemokine production, and pro-inflammatory cytokine production and involvement of cytokine-inducible DNA-binding proteins like signal transducers and activators of transcription (STAT) proteins, latent transcription factors [4]. The synthesis and release of cytokines upon inflammatory action mainly medicated by oxidative stress mechanisms like mitogen activated protein kinases (MAPKs) and nuclear factor-κ B (NF-κB) [4].

Free oxygen radicals and pro-inflammatory cytokines which are released by neutrophils and macrophages during acute pancreatitis,

exacerbate the inflammatory response by causing an increase in local and systemic capillary permeability and by promoting leukocyte adhesion and extravasation [3]. The cellular mechanism of pro-inflammatory cytokines is regulated by nuclear factor-κB (NF-κB) and activator protein-1 (AP-1). NF-κB and AP-1 are two transcriptional complexes required for the early response gene expression of inflammatory molecules [3]. It has been reported that interleukins IL-1b, IL-6, IL-8, and TNF-α levels are significantly increased in both sera and tissues of patients with acute pancreatitis [3]. The main objective of this review is to collect the information about the role of pro-inflammatory cytokines in the development of acute pancreatitis.

Pathophysiology of acute pancreatitis

Premature activation of digestive enzymes in acinar cells during acute pancreatitis results in auto-digestion of the pancreas. Trypsinogen, a serine protease, is now thought to be the first enzyme to be activated; subsequently other digestive enzymes (chymotrypsinogen and proelastase) are cleaved and activated [5-8]. The pancreas has a variety of self-protective mechanisms to prevent intracellular zymogen activation and subsequent auto-digestion. However in acute pancreatitis, these protective mechanisms are no longer effective or are overwhelmed [5-8]. As a result, the activated pancreatic enzymes destroy the pancreatic cell membranes and subsequently the entire pancreatic tissue, causing pancreatic oedema, vascular damage, haemorrhage, and necrosis. Strong local inflammatory response activates leukocytes and endothelial cells among others. Secreted bioactive molecules from infiltrating leukocytes contribute to local damage and subsequently to the systemic inflammatory response, which may result in multiple organ dysfunction and ultimately death [9]. A number of inflammatory mediators have been implicated in the recruitment of leukocytes into the pancreas [9]. Inflammatory mediators such as substance P and chemokines along with cytokines, interleukins, intercellular adhesion molecules and platelet activating factor have been shown to play significant roles in the pathogenesis of acute pancreatitis [9-11].

Role of substance P in the pathogenesis of acute pancreatitis

Substance P is an 11-amino acid neuropeptide that was originally isolated and purified by Chang and Leeman from bovine pituitary glands [12]. It is a member of the tachykinin family and has been shown to induce rapid smooth muscle contraction in guinea pig ileum and rat duodenum [12].

Studies have indicated that substance P by acting through neurokinin 1 receptor (NK1R) stimulates pancreatic acinar cells to release chemokines MCP-1, MIP-1a, MIP-2 and RANTES by PLC- dependent mechanisms (Figure 1). The released chemokines acting via chemokine receptors facilitate the release of cytokines (IL-1, IL-8 and TNF-α) as shown in Figure 2, which play an important role in the pathogenesis of acute pancreatitis [11,13-15]. Both genetic deletion of NK1R and blockade of NK1R with its selective antagonist CP96345, protected mice against acute pancreatitis and associated lung injury [11,16]. Substance P has also been shown to play an important role in the pathogenesis of asthma, inflammatory bowel disease and arthritis [17,18]. Substance P levels in the pancreas and NK1R expression on pancreatic acinar cells are increased during secretagogue-induced experimental pancreatitis [11,14,15,19]. It has been suggested that the neuropeptide substance P plays an important role in the evolution of pancreatic inflammatory diseases such as acute pancreatitis [11]. These observations indicate that substance P, acting through NK1R, plays an important pro-inflammatory role in regulating the severity of acute pancreatitis. Substance P also induces an increase in $[Ca^{2+}]_i$ which results in the phosphorylation of PKCα/βII, ERK and JNK; leading to the activation of NF-κB p65, AP-1, c-Jun and ultimately to chemokine production [20]. However, the exact mechanism(s) by which substance P contributes to the pro-inflammatory signalling in acute pancreatitis is not completely understood [20].

Role of chemokines in the pathogenesis of acute pancreatitis

Chemokines are a family of small (8-10 kDa) inducible cytokines with activating and chemotactic effects on leukocyte subsets. These proteins are defined by four invariant cysteine residues and are classified into four sub-families (two major and two minor) based on the relative position of the first two cysteine residues: CXC (α-subfamily), CC (β-subfamily), C (γ-subfamily) and CX3C (δ-subfamily) chemokines (C stands for cysteine and X stands for other amino acids) [21].

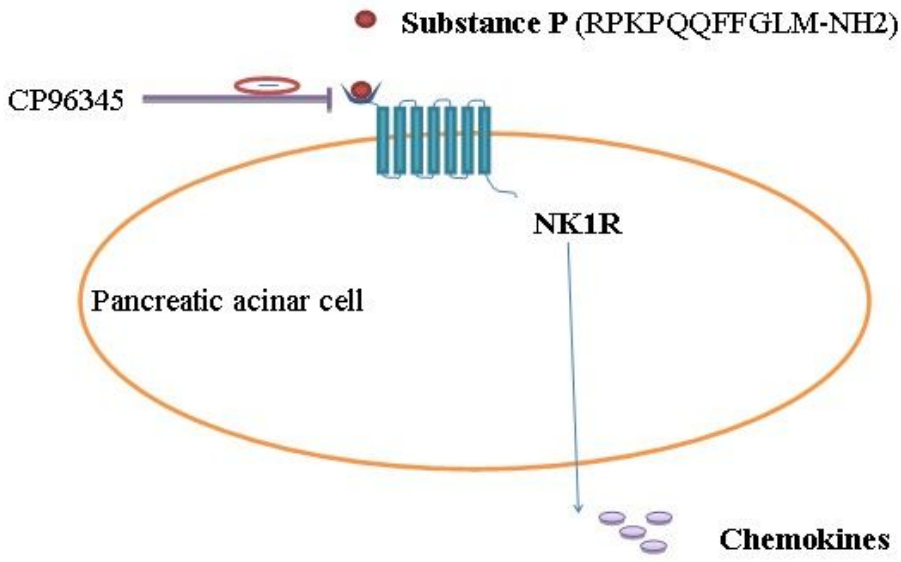

Figure 1: Induction of chemokines by substance P in the pancreatic acinar cells

The two major subfamilies CC and CXC chemokines have been extensively investigated in various disease conditions including acute pancreatitis.

Chemokines act as regulators of immune, inflammatory and hematopoietic processes. They play a major role in leukocyte trafficking, recruitment and recirculation. The CC chemokine family members include monocyte chemoattractant protein- 1 (MCP-1), MCP-2, macrophage inflammatory protein; MIP-1a, MIP-1b, RANTES, eotaxin and thymus- and activation-regulated chemokine (TARC). The CC chemokines serves as chemoattractant for monocytes/macrophages, activated T-cells, B-cells, eosinophils, basophils and dendritic cells. [22,23].

A study of chemokine gene expression in rat pancreatic acinar cells showed an up-regulated rat CXC chemokine mob-1 and CC chemokine MCP-1 mRNA expression within 1 h of cerulein-induced acute pancreatitis *in vivo*. The mob-1 mRNA was also induced by either retrograde injection of bile salts or cerulein in acinar cells *in vitro* [24,25]. An *in vitro* study on cholecystokinin (CCK)- and ethanol-treated rat pancreatic acinar cells demonstrated that rat pancreatic acinar cells secreted MCP-1 and RANTES in response to CCK and ethanol stimulation, suggesting a role for these two chemokines in the pathogenesis of acute pancreatitis [26]. It has also been shown that cerulein hyper-stimulation induced synthesis of MCP-1 but not CINC in rat pancreatic acinar cells [9]. This MCP-1 synthesis is mediated through a calcium-dependent mechanism involving NFκB activation.

The role of MCP-1 as well as two other CC chemokines MIP-1α and MIP-1β has been extensively evaluated in human acute pancreatitis. It was found that complicated acute pancreatitis is associated with significantly elevated levels of local and systemic concentrations of MCP-1 and MIP-1α [27]. A close correlation between the severity of remote organ failure and the degree of MCP-1 elevation suggests that MCP-1 might play a pivotal role in the pathological mechanism of complicated human acute pancreatitis [27]. MCP-1 is believed to contribute to the progression of chronic pancreatitis which results from repetitive pancreatic injury with sustained production of various pro-inflammatory cytokines and chemokines through monocyte/macrophage recruitment [28]. Moreover, it has been reported that blockade of MCP-1 could reduce the development of pancreatic fibrosis in chronic pancreatitis [29].

Role of nuclear factor κB (NFκB) in the pathogenesis of acute pancreatitis

NFκB activation is a key mediator of the inflammatory response in acute pancreatitis [30-33]. It is an ubiquitous transcription factor which is implicated in the regulation of many genes that code for mediators of the immune and acute phase of inflammatory responses [34]. In a classical pathway, NFκB is sequestered in the cytoplasm of most resting cells through its association with an inhibitory protein called IκB. During stimulation by IL-1 or TNF-α, a whole cascade of adaptor proteins and protein kinases are activated, leading to the phosphorylation of IκB by the IκB kinases α and β (IKK α/β) [35].

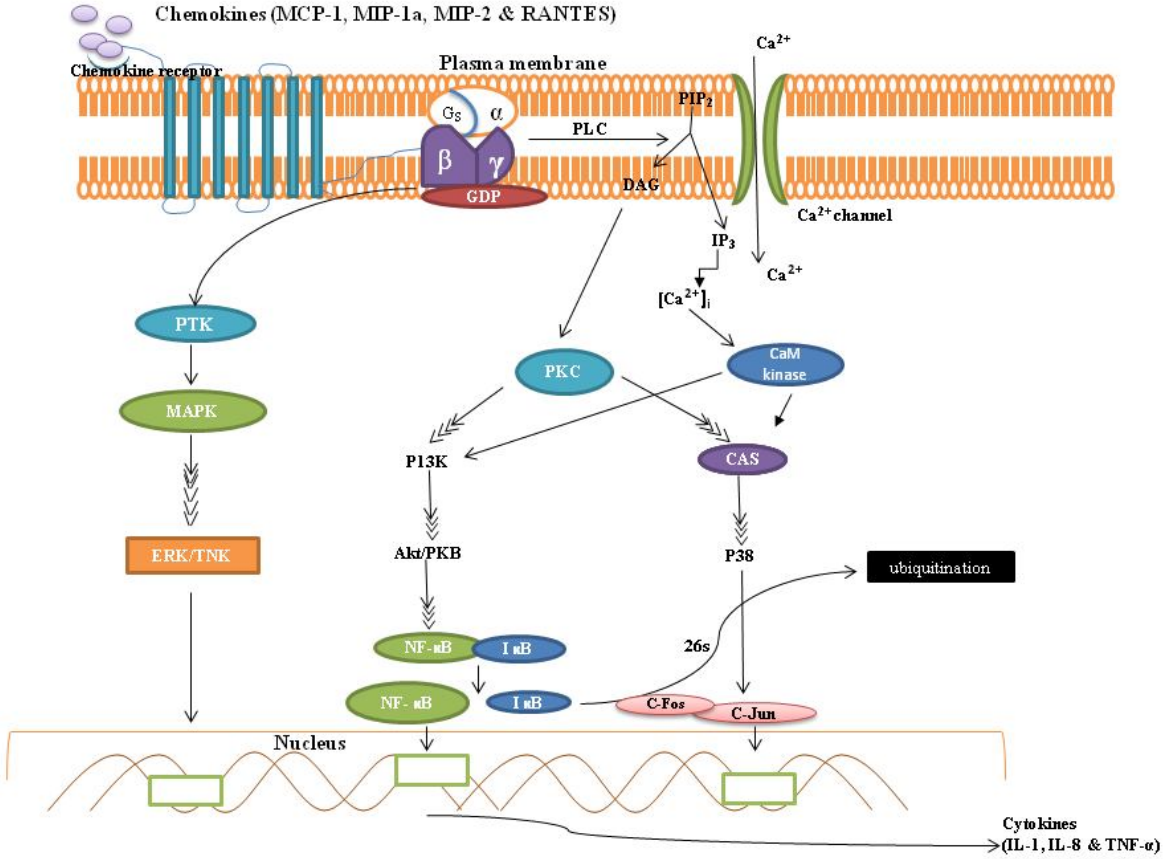

Figure 2: Downhill mechanism of cytokines secretion, as a result of chemokine receptor activation. CAS: Crk-associated substrate; ERK- Extracellular signal-regulated kinases; PTK- Protein tyrosine kinases; CaM kinase, Ca^{2+}/calmodulin-dependent kinases

This depends on the regulatory protein NEMO/ IKK γ (NFκB essential modifier) associated with the complex containing two kinases, IKK α and IKK β [36,37].

Once phosphorylated, IκB is ubiquitinated and subsequently degraded through 26S proteasome. Consequently, NFκB is freed to migrate into the nucleus, and binds to its consensus decameric sequence located in the promoter region of several genes involved in the pro-inflammatory response, encoding various immunoreceptors, cell adhesion molecules, cytokines and chemokines [34].

The first report in 2005 by Meng *et al*, demonstrated the effect of resveratrol on nuclear factor Kappa-B (NF-κB) and the inflammatory response in a rat model of severe acute pancreatitis. The rats treated with resveratrol were reported to have significantly lower levels of NF-κB, Tumour Necrosis Factor-α (TNF-α), and Interleukin-8 (IL-8) expressions compared to control animals. Moreover, histological examination of the pancreas showed less hyperaemia, necrosis, and oedema in the treated group [38].

Role of activator protein-1 (AP-1) in the pathogenesis of acute pancreatitis

AP-1 expression is induced by multiple stimuli such as inflammatory cytokines, mitogenic growth factors, phorbol esters, oncogenes and cellular stress among others. It is activated during the cell cycle to promote cell survival, differentiation and adaptive responses.

NF-κB and AP-1 regulate many gene expressions which participate in immune and inflammatory responses. NF-κB is found in an inactive form in the cell cytoplasm attached to inhibitor protein kappa B (IκB). When activated, IκB is phosphorylated by specific IKK kinases and rapidly degraded though proteasome-dependent pathways. The other transcription factor, AP-1 is a homo- or heterodimer complex molecule formed from Jun, Fos or the activating transcription factor subunits [3]. AP-1 is also involved in tissue proliferation, differentiation and transformation as observed in adult tissues and plays a key role in the regulation of the inflammatory process. AP-1 can be activated by growth factors, cytokines, chemokines, hormones and multiple environmental factors [3].

Mitogen-activated protein kinases (MAPKs)

MAPKs are a family of serine/threonine kinases activated by a cascade of intracellular phosphorylation events and transduce signals from the cell surface to the nucleus [39,40]. There are three well-characterized sub-families of MAPKs that control an array of physiological processes. It is generally believed that ERKs function in the control of cell division and Jun- N terminal kinases (JNKs) are critical regulators of transcription and p38 MAPKs are activated by inflammatory cytokines and environmental stress [41].

CONCLUSION

The current review describes the various inflammatory mediators that influence the pathogenesis of acute pancreatitis. Acinar cell injury by trypsinogen activation plays a critical role in the pathogenesis mechanism of acute pancreatitis. The severity of cell death depends on the level of both pro-inflammatory and inflammatory mediators which involve the development of both apoptosis and necrotic pancreatic cell death. It is hoped that this review will assist investigators to better understand the mechanisms involved in the pathogenesis of acute pancreatitis and thus facilitate the development of more effective therapeutic molecules to treat acute pancreatitis.

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