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Special Article

Apoptosis of pancreatic acinar cells in acute pancreatitis: is it good or bad?

M. Bhatia

Department of Pharmacology, National University of Singapore, Singapore

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Abstract

Acute pancreatitis is a disease of variable severity in which some patients experience mild, self-limited attacks while others manifest a severe, highly morbid, and frequently lethal attack. The events that regulate the severity of acute pancreatitis are, for the most part, unknown. Several recent studies have suggested that the acinar cell response to injury may be an important determinant of disease severity. In these studies, mild acute pancreatitis was found to be associated with extensive apoptotic acinar cell death while severe acute pancreatitis was found to involve extensive acinar cell necrosis but very little acinar cell apoptosis. These observations have led to the hypothesis that apoptosis might be a favorable response to acinar cell and that interventions which favor induction of apoptotic, as opposed to necrotic, acinar cell death might reduce the severity of an attack of acute pancreatitis. This review aims to discuss our current understanding of the contribution of acinar cell apoptosis to the severity of acute pancreatitis.

Keywords: apoptosis - necrosis - inflammation - pancreatitis

* Correspondence to: Madhav BHATIA, Ph.D.
Department of Pharmacology,
National University of Singapore, Faculty of Medicine,
Bldg MD2, 18 Medical Drive, Singapore 117597.
Tel.: (65)-6874-8256, Fax: (65)-6873-7690
E-mail: mbhatia@nus.edu.sg

Introduction

Acute pancreatitis is a common clinical condition, whose incidence has been increasing over recent years [1,2]. In the majority of patients the condition is mild but about 25% of patients suffer a severe attack and between 30 to 50% of these will die [3-5]. Most cases are secondary to biliary disease or excess alcohol consumption. The events that regulate the severity of acute pancreatitis are, for the most part, unknown. The exact mechanisms by which diverse etiological factors induce an attack are still unclear, but once the disease process is initiated common inflammatory and repair pathways are invoked. There is a local inflammatory reaction at the site of injury, if marked this leads to a systemic inflammatory response syndrome (SIRS), and it is this systemic response that is believed to be ultimately responsible for the majority of the morbidity and mortality [6-12]. Several recent studies have, however, suggested that acinar cell response to injury may, itself, be an important determinant of disease severity. In this review, I intend to discuss our current understanding of the contribution of acinar cell injury – in particular, acinar cell apoptosis – to the severity of acute pancreatitis.

Apoptosis

Apoptosis was defined originally as a physiological or programmed form of cell death that affects scattered cells in a tissue, and has a characteristic and stereotypical morphology, including cell shrinkage, retention of organelles and nuclear chromatin condensation which occur in response to a variety of stress-related stimuli. Apoptosis is defined by distinct morphological and biochemical changes mediated by a family of cysteine aspartases (caspases), which are expressed as inactive zymogens and are proteolytically processed to an active state following an apoptotic stimulus. Early studies [13] first elucidated the existence of a genetically controlled cell death program in which at least three gene products, CED-3, CED-4, and CED-9, participate to cause selective programmed cell death during *Caernohabditis elegans* development. Subsequent studies in other organisms revealed that several cysteine proteases that share homology to CED-3 are present in mammalian cells. Fourteen such cysteine proteases have been identified so far, and they are identified as caspase-1 to caspase-14 [5,14,15].

Caspases are the molecular executioners of apoptosis because they bring about most of the morphological and biochemical characteristics of apoptotic cell death. They are a family of constitutively expressed proenzymes that undergo proteolytic processing to generate its activated form [5,14,15]. During apoptosis, the effector caspases (such as caspases-3, -6, and -7) cleave numerous proteins located in the cell membrane, nucleus, and cytoplasm. The activation of caspase-activated DNAse (CAD) to facilitate DNA degradation, cleavage of nuclear lamins to facilitate nuclear shrinkage and budding, and activation of p21-activated kinase 2 to cause active blebbing in apoptotic cells are a few of the important functions mediated by caspases in the apoptotic process [5].

Two separable pathways leading to caspase activation have been characterized [5,16,17]. The extrinsic pathway is initiated by ligation of transmembrane death receptors (CD95, TNF receptor, and TRAIL - TNF-related apoptosis-inducing ligand - receptor) to activate membrane-proximal (activator) caspases, which in turn cleave and activate effector caspases. The intrinsic pathway requires disruption of the mitochondrial membrane and the release of mitochondrial proteins and cytochrome c. Cytochrome c functions with apoptotic protease activating factor-1 (Apaf-1) to induce activation of caspase-9, thereby initiating the apoptotic caspase cascade [5,17,18].

The release of cytochrome *c* by mitochondria is almost a universal feature found in response to various intracellular stimuli, including DNA damage, glucocorticoids, oxidative injury, and growth factor deprivation, although they may not play a significant role in receptor-mediated apoptosis [5,19]. Although classically considered the powerhouses of the cell, it is now understood that mitochondria are also "gatekeepers" that ultimately determine the fate of the cell. The mitochondrial decision as to whether a cell lives or dies is complex, involving protein-protein interactions, ionic changes, reactive oxygen species, and other mechanisms that require further elucidation. Once the death process is initiated, mitochondria undergo conformational changes, result-

ing in the release of cytochrome *c*, caspases, endonucleases, and other factors leading to the onset and execution of apoptosis [5,19]. The activation of caspase-9 is mediated by a macromolecular complex, the apoptosome, that is formed in response to a cellular commitment to apoptotic death. Formation of the apoptosome is initiated upon release of certain mitochondrial proteins, such as cytochrome *c*, from the mitochondrial intermembrane space. Released cytochrome *c* binds to monomers of Apaf-1 in the cytosol, inducing a conformational change that enables stable association with (deoxy)adenosine triphosphate. Apaf-1 monomers then assemble into the heptameric apoptosome, which in turn binds to procaspase-9. Once recruited, procaspase-9 acquires catalytic competency, is proteolytically cleaved, and activates the effector caspases, a process that culminates in apoptotic cell death [5,20].

Mitochondrial membrane permeabilization, that leads to the release of pro-apoptotic mitochondrial proteins including cytochrome *c*, is regulated by the opposing actions of pro- and anti-apoptotic Bcl-2 family members. The ever-growing mammalian Bcl-2 family of apoptotic regulators shares homology with the *C. elegans* antiapoptotic molecule CED-9 [5,21]. Based on their structure and functional similarities, Bcl2 family members are divided into the proapoptotic (Bax, Bak, and Bok) and antiapoptotic (Bcl2, $BclX_L$, Bcl-w, Mcl-1, and A1) groups [5,22]. A third class of death effector molecules sharing homology only to the Bcl-2 homology-3 (BH3) domain can activate proapoptotic Bcl2 family members or inactivate antiapoptotic members [5,23].

There is considerable cross-talk between the extrinsic and intrinsic pathways. For example, caspase-8 can proteolytically activate Bid, which can then facilitate cytochrome *c* release [5]. This apparently amplifies the apoptotic signal following death receptor activation, and different cell types may be more reliant on this amplification pathway than others. Conversely, activators of the intrinsic pathway can sensitize the cell to extrinsic death ligands. Although the extrinsic and intrinsic signals are considered to take two distinct pathways to execute cell death, receptor-initiated cell death can involve the mitochondrial pathway through the BH3-only protein Bid. It should be noted, however, that the cross talk between the two pathways is minimal under most conditions.

Apoptosis and necrosis in acute pancreatitis

Human as well as experimental acute pancreatitis is characterized by progressive cell death, the mechanisms of which remain poorly understood. Necrosis has classically been considered the major form of cell death in acute pancreatitis [24,25], whereas apoptosis was suggested to mediate atrophy in the organ [26,27]. However, careful biochemical and morphological examination of experimental models of acute pancreatitis has shown that severe acute pancreatitis (e.g. that induced by pancreatic duct ligation in the opossum, by choline-deficient and ethionine supplemented diet in the mouse, and by caerulein-hyperstimulation in the mouse) is associated primarily with necrosis but little apoptosis, whereas mild acute pancreatitis (e.g. that induced by pancreatic duct ligation and by caerulein-hyperstimulation in the rat) is associated primarily with apoptotic cell death and little necrosis [28,29]. In other words, the severity of acute pancreatitis is inversely related to the extent of acinar cell apoptosis (Fig. 1).

Caspase activation and acinar cell apoptosis

Caspase activation may be one of the mechanisms of apoptosis in acute pancreatitis. In fact, there are studies showing that several pathogenic factors in acute pancreatitis lead to an activation of caspases in acinar cells. According to one report, stimulation of isolated rat pancreatic acini with CCK, which serves as a model for human acute edematous pancreatitis, leads to a rapid redistribution and activation of caspase 8 [30]. Caspase 8 is regarded as an early regulator of a proteolytic cascade leading to apoptosis. Besides initiator caspase, the activation of executioner caspase is also reported during acute pancreatitis. A recent paper has reported that oxidative stress, a major pathogenic factor in acute pancreatitis, induces

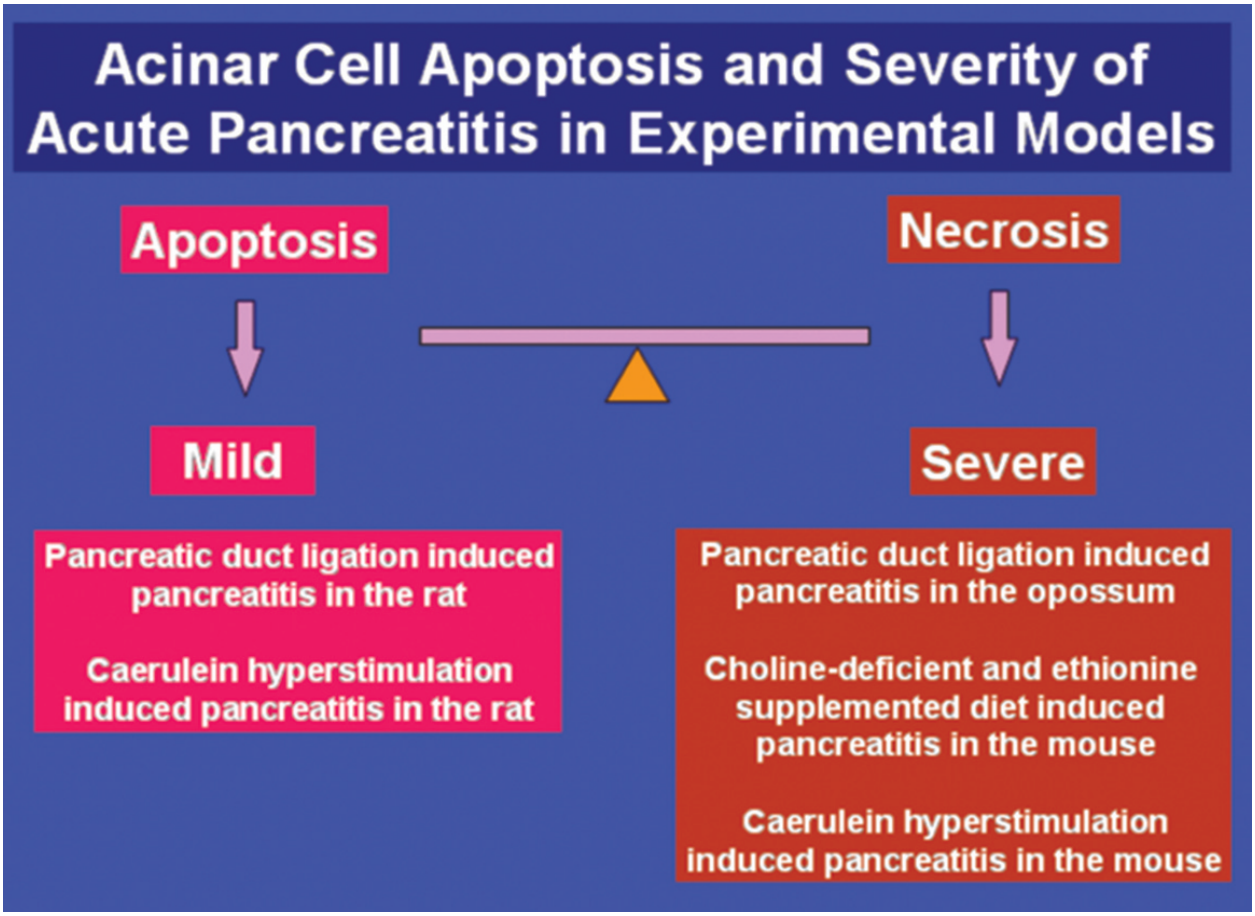

Fig. 1 The severity of acute pancreatitis is inversely related to the extent of pancreatic acinar cell apoptosis in different experimental models of acute pancreatitis.

apoptosis of acinar cells involving activation of caspase-3, which degrades the DNA repair protein Ku70 and Ku80 [31]. Another report has shown that cholecystokinin (CCK) stimulates death signaling pathways in rat pancreatic acinar cells, including caspase activation, cytochrome c release, and mitochondrial depolarization, leading to apoptosis. The mitochondrial dysfunction is mediated by upstream caspase(s). CCK causes mitochondrial alterations through both PTP (permeability transition pore)-dependent (cytochrome c release) and PTP-independent (mitochondrial depolarization) mechanisms. In addition to apoptosis, caspases also regulate other processes in the pancreatic acinar cell that play key roles in pancreatitis; in particular, caspases negatively regulate necrosis and intra-acinar cell activation of trypsin [32]. This may explain the inverse correlation between the extent of apoptosis on the one hand and necrosis and the severity of the disease on the other hand observed in experimental models of pancreatitis.

Apoptosis associated genes expression and acinar cell apoptosis

In addition to caspase, the Bcl-2 family of apoptotic regulators is another one of the functional components comprised in the apoptosis pathway [5]. Recent studies have shown that acinar cell apoptosis observed in acute pancreatitis is also at least partly attributable to the greatly increased pro-apoptotic bax gene expression. Gomez et al reported that acute pancreatitis induced a prompt increase in pancreatic bax mRNA levels closely followed by an increase in bax protein levels in the acinar cells

and they suggested that acinar cell apoptosis observed during caerulein-induced acute pancreatitis is attributable, at least in part, to the greatly increased bax expression [33].

Besides Bcl-2 family, SIP gene has recently been identified, whose expression localized in acinar cell, and demonstrated that as a stress-inducible gene, it is overexpressed during pancreatitis and promoted cell apoptosis [34].

Transcription factors in acinar cell apoptosis

Transcription factors may be involved in the signal transduction pathways leading to apoptosis. For example, the transcription factor p53 plays a role in the initiation of apoptosis by inducing bax expression. A possible role of this factor in acinar cell apoptosis, however, remains unclear. In a study [33], it was reported that pancreatic p53 mRNA levels are temporally coordinated with those of bax during acute pancreatitis. However, another study reported that the mechanisms of pancreatic acinar cell apoptosis correlated with the expression of apoptosis-regulated gene bax, but had no relationship with the expression of p53 [35].

Other transcription factors, such as nuclear factor kappa B (NF–κB) and activator protein (AP)-1, may also be involved in the signal transduction pathways leading to apoptosis. Earlier studies have suggested a possible link between apoptosis and activation of NF-κB in pancreatic acinar cells [36]. And the potential role of NF-κB in cell death was suggested by activation of TNF-α transcription [36].

Neutrophils and apoptosis

Neutrophils activation is an important determinant of severity of acute pancreatitis. Recently, some reports have indicated that the depletion of neutrophils results in a significant increase in acinar cells undergoing apoptosis. Sandoval et al. have reported a dramatic increase in apoptotic acinar cells by administration of antineutrophil serum, which, comparable to aICAM-1 (an ICAM-1 neutralizing antibody), significantly reduced the extent of necrosis and the inflammatory infiltrate [37]. Yet another study, using taurocholate-induced severe acute pancreatitis as the experimental model, showed that neutrophils, via a TNF-α-dependent mechanism, may be involved in the development of apoptotic as well as necrotic forms of acinar cell death [38].

Cytokines and apoptosis

In addition to their role in inducing inflammation, cytokines, such as TNF-α or interleukin-1β, which are released by neutrophils (and to a lesser extent, by acinar cells) have recently been shown to induce apoptosis in pancreas [39]. Pancreatic acinar cells have been reported produce and release TNF–α [39]. These authors found that TNF-α was involved in the development of pancreatitis, and it mediated apoptosis in acinar cell suspension in vitro and also in vivo in the caerulein model of acute pancreatitis [39].

Induction of pancreatic acinar cell apoptosis by agents that do not cause acute pancreatitis

CCK is an inducer of pancreatic acinar cell apoptosis. It can, however, also induce acute pancreatitis. In addition to CCK, other inducers of pancreatic acinar cell apoptosis have been used to investigate cell death in relation to acute pancreatitis. Examples of these compounds are menadione and crambene (1-cyano-2-hydroxy-3-butene-CHB). Menadione is a quinone that is metabolized by flavoprotein reductase to semiquinone, which can be oxidized back to quinone in the presence of molecular oxygen. In this redox cycle the superoxide anion radical, hydrogen peroxide and other reactive oxygen species are generated [40]. Menadione can cause elevations in the cytosolic Ca^{2+} concentration contributing to cell death [41]. In a recent study, menadione has been shown to evoke repetitive cytosolic Ca^{2+} spikes, partial mitochondrial depolarization, cytochrome *c* release and apoptosis of pancreatic acinar cells *in vitro* [41]. Crambene is a stable plant

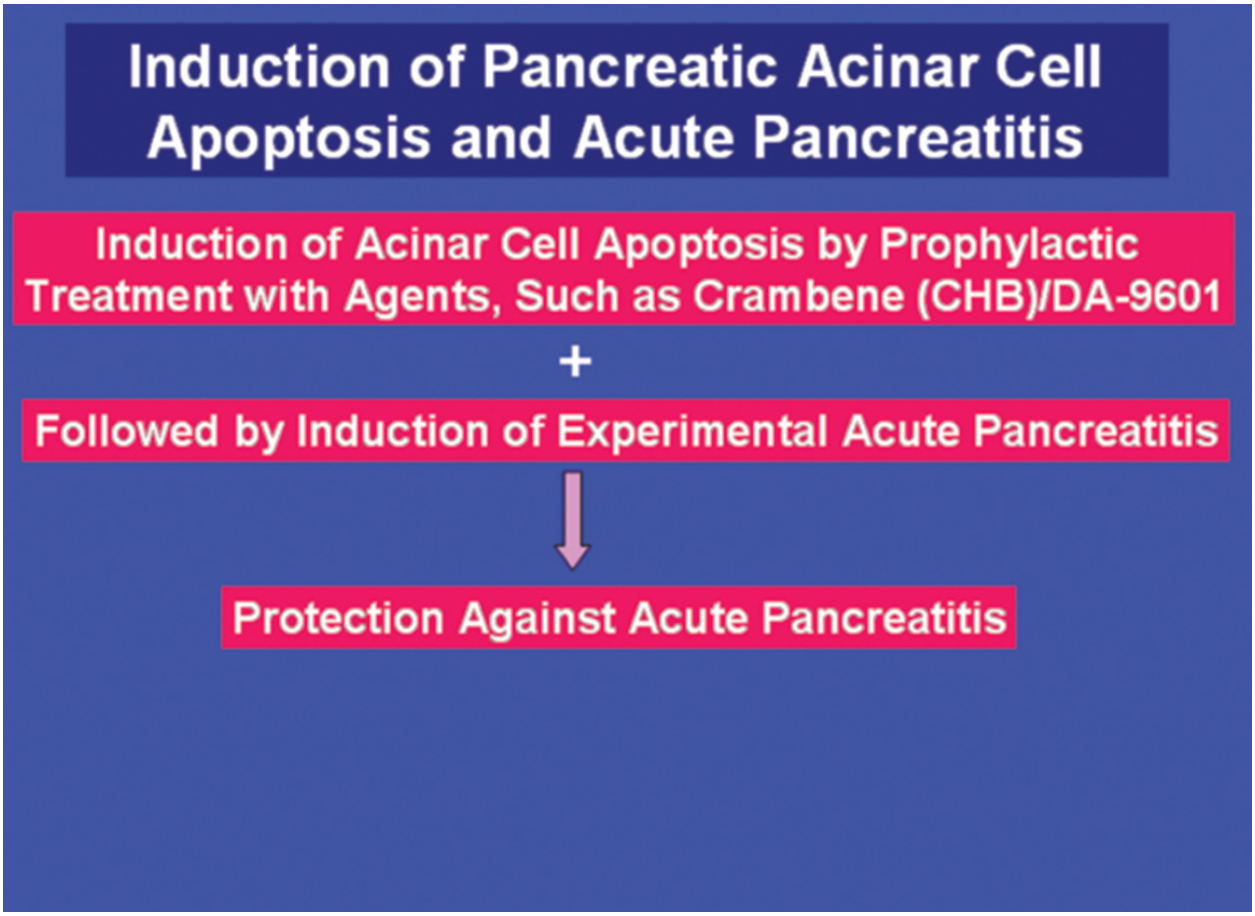

Fig. 2 Is acinar cell apoptosis good or bad - upon prophylactic induction, it has been shown to be protective against acute pancreatitis.

nitrile found in many cruciferous vegetables. Crambene is a potent inducer of "phase II" detoxification enzymes, including certain glutathione S-transferases and quinone reductase, which are important enzymes associated with conjugation and elimination of reactive chemical intermediates and carcinogens [42,43]. Also, it has been shown to cause cell cycle arrest of the Hep G2 cells or colonic adenocarcinoma cells in G2/M phase [41]. We have earlier shown that upon intravenous administration, crambene induces apoptosis of the pancreatic acinar cells [9,10]. The mechanism of induction of pancreatic acinar cell apoptosis by crambene is not yet clear. However, induction of pancreatic acinar cell apoptosis with crambene protects mice against acute pancreatitis induced by caerulein [45,46]. This is despite the fact that crambene does not alter the interaction of CCK to its receptor on pancreatic acinar cells [45]. The maximal protective effect of crambene against acute pancreatitis is observed when the pancreatic acinar cells have become committed to apoptosis [45,46]. Studies are in progress to investigate the mechanism of induction of pancreatic acinar cell apoptosis by crambene. A possible role of the gap junction communication (GJC) and connexin gene Cx32 in crambene-induced apoptosis has been suggested by a recent study [47]. Mice deficient in Cx32 gene were found to be resistant to apoptosis induced by crambene and were more susceptible to acute pancreatitis than their wild-type counterparts [47]. Although the role of GJC in apoptosis is still unclear, the data in this study [47] indicate that defective GJC in Cx32-deficient mice prevented acinar cell apoptosis at a step upstream of caspase-3 activation. A protective effect of induction of pancreatic acinar cells against acute pancreatitis was substantiated in another study in which it was

shown that induction of apoptosis by an extract of *Artemisia asiatica* (DA-9601) was beneficial in caerulein-induced pancreatitis in rats [48].

Conclusion

Both apoptotic and necrotic forms of cell death are seen in clinical, as well as experimental acute pancreatitis. The mechanisms of pancreatic acinar cell death - apoptosis and necrosis - in acute pancreatitis are just beginning to be identified. The extent of pancreatic acinar cell apoptosis has been shown to be inversely related to the severity of the disease, suggesting that apoptosis is a teleologically beneficial form of cell death in acute pancreatitis. Indeed, in an experimental setting, induction of apoptosis with the plant nitrile crambene has a protective action against acute pancreatitis. However, a protective effect of induction of acinar cell apoptosis against acute pancreatitis has been observed only prophylactically (Fig. 2). Active research in this direction will facilitate a transition of our knowledge of necrosis vs. apoptosis of pancreatic acinar cells from bench to bedside.

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