Antiproteases and the Pancreas: Basic and Clinical Update.
Conclusion Remarks

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What Is the Target of a Protease Inhibitor?
There is no proven specific drug therapy for the treatment of acute pancreatitis, including protease inhibitors. In this virtual Round Table, our invited authors reviewed the past, present, and projected future clinical relevance of protease inhibitors. Through their discussion, protease inhibitors are demonstrated to have broad inhibitory actions on serine proteases, the coagulation system, the complement system and the production of pro-inflammatory cytokines, both in vitro and in vivo. We are assured that proteases remain an important and active field of study.

Several protease inhibitors, including gabexate mesilate, nafamostat mesilate and ulinastatin, have been used for the treatment of acute pancreatitis in Japan [1, 2]. Camostat mesilate, an orally active protease inhibitor, has also been used for the treatment of chronic pancreatitis [2]. Their major action in suppressing pancreatitis is that of inactivating trypsin and preventing autodigestion. In recent years, several actions of a protease inhibitor have been demonstrated in vitro and in vivo studies. For example, gabexate has been revealed to inhibit nuclear factor-kappaB (NF-kappa B) activation in human monocytes or human umbilical vein endothelial cells (HUVECs) [3, 4]. NF-kappa B plays a crucial role in inflammation, immunity, cell proliferation, and apoptosis [5, 6]. Therefore, gabexate has been hypothesized to have various functions in the pathogenesis of acute pancreatitis, chronic pancreatitis and pancreatic cancer, if gabexate therapies target NF-kappa B.

Protease Inhibitors and Acute Pancreatitis
Protease inhibitors can block premature trypsin activation, but they were not as effective clinically as expected for the treatment of acute pancreatitis [7, 8, 9]. An inappropriate conversion of pancreatic zymogens to active enzymes within the pancreatic parenchyma was hypothesized to initiate the inflammatory process [5]. Cellular events leading to pancreatitis involve an inflammatory cascade with premature activation of trypsin in acinar cells. Trypsin activates a subset of enzymes, leading to the release of cytokines from acinar cells and the recruitment of inflammatory cells. Although the exact mechanisms which trigger the inflammation and necrotizing process are not completely understood, activated leukocytes play an important role in the pathogenesis of acute pancreatitis. The initial phase of severe acute pancreatitis depends on neutrophil activation, accompanied by a systemic inflammatory response syndrome (SIRS). Pro-inflammatory cytokines including interleukin (IL)-1beta, IL-6, IL-8, tumor necrosis factor-alpha (TNF-alpha), platelet-activating factor (PAF), and the anti-inflammatory cytokines IL-2 and IL-10, were implicated in the pathogenesis of SIRS in acute pancreatitis [10, 11]. This understanding
has led to the development of alternative treatment strategies aimed at interrupting the inflammatory response and reducing the degree of SIRS and multiple organ dysfunction.

Dr. Chen [12] demonstrated that protease inhibitors could modulate inflammatory cytokine responses in experimental pancreatitis. Therapies which target cytokines have been studied; anti-inflammatory agents such as lepixafant are not sufficient to ameliorate SIRS in severe acute pancreatitis. Further research into novel anti-inflammatory mediator therapies are warranted [13].

Another candidate for target therapy by protease inhibitors could be protease-activated receptor-2 (PAR-2). PAR-2 is a widely expressed ligand receptor which can be activated by trypsin and other trypsin-like serine proteases [14, 15]. PAR-2 is widely expressed in the gastrointestinal tract and is also abundantly expressed in the pancreas. PAR-2 is expressed in pancreatic acinar cells and the luminal side of the pancreatic duct cell. Both PAR-1 and PAR-2 were reported to be expressed in pancreatic satellite cells, vascular endothelial cells and vascular smooth muscle cells in the pancreas. In the exocrine pancreas, PAR-2 activation has been found to accelerate acinar cell secretion of digestive enzymes and to alter duct cell ion channel function. PAR-2 may have a dual role in acute pancreatitis: protecting acinar and duct cells against pancreatitis-induced cell damage and/or aggravating the systemic complications of acute pancreatitis, which are the major cause of mortality in the early phase of necrotizing pancreatitis [16]. Further studies are required to reveal whether protease inhibitor therapy targeting PAR-2 have a beneficial or a harmful effect on the exacerbation of acute pancreatitis.

Protease inhibitors administered intravenously are unlikely to reach the pancreas because of their pharmacokinetics and impaired microcirculation in an inflamed pancreas. Protease inhibitors were not as effective as expected because of the timing of the administration, the concentration of the protease inhibitor in pancreatic tissue and the diminution of the vasculature of the pancreas [2]. To increase the concentration of the protease inhibitor, arterial infusion of the protease inhibitor in acute necrotizing pancreatitis was conducted [17]. Continuous regional arterial infusion (CRAI) of protease inhibitors and antibiotics has been used in Japan [1]. Dr. Takeda [18] reported excellent results in clinical studies on the efficacy of CRAI therapy in severe acute pancreatitis. However, randomized controlled trials in multiple centers are necessary to justify CRAI therapy of protease inhibitors in the early stage of acute pancreatitis and to recommend it as a standard of care. The pancreas is susceptible to ischemic insult, which can exacerbate acute pancreatitis. There is also increasing evidence of pancreatic and systemic microvascular disturbances in the pathogenesis of pancreatitis, including vasoconstriction, shunting, inadequate perfusion, and increased blood viscosity and coagulation [19, 20, 21, 22]. These processes may be caused or exacerbated by ischemia-reperfusion injury and the development of oxygen-derived free radicals. Acute pancreatitis impairs the pancreatic and systemic microcirculation, which is a key pathological process in the development of severe necrotizing disease. Therapies targeted at mediators of microvascular changes in acute pancreatitis, such as endothelin 1, platelet-activating factor (PAF), and intercellular adhesion molecule (ICAM) 1 are currently being investigated [20]. Gabexate was reported to improved microcirculatory environment after induction of experimental acute pancreatitis [23]. Moreover, gabexate was seen to regulate NF-kappa B and inflammatory cytokines, which could have an effect on microcirculation, vascular permeability and coagulation in acute pancreatitis.

Because multiple cascades which independently alter the course of acute pancreatitis (protease activation, inflammatory cytokines, oxidant stress, and apoptosis), it is unrealistic to expect that blocking a single cascade will dramatically abort human acute pancreatitis. However, inhibition of protease activation may certainly
constitute an important arm of a multi-drug approach to acute pancreatitis.

**Protease Inhibitor and ERCP-Induced Pancreatitis**

The potential benefits of protease inhibitors in preventing post-endoscopic retrograde cholangiopancreatography (ERCP) acute pancreatitis have been frequently discussed [24, 25, 26]. Several studies have demonstrated that the prophylactic administration of protease inhibitors is of significant value [26]. Dr. Tsujino et al. [27] focused on cost-effectiveness and the prolonged administration of gabexate compared with ulinastatin after ERCP. Prolonged infusions for pharmacologic prophylaxis against severe pancreatitis after ERCP may need an additional hospital stay. A short-term infusion of ulinastatin is recommended in preventing post-ERCP pancreatitis in high-risk patients. It is unclear whether all patients undergoing ERCP would benefit from the use of protease inhibitors or only those who are at greater risk for pancreatitis. Magnetic resonance cholangiopancreatography (MRCP) is now available for the diagnosis of pancreatic diseases; unnecessary ERCPs should be avoided in routine practice [28].

**Protease Inhibitors and Chronic Pancreatitis**

Mutations in the protease serine type 1 (PRSS1) gene encoding cationic trypsinogen play a causative role in chronic pancreatitis [29, 30]. It has been shown that the PRSS1 mutations increase autolytic conversion of trypsinogen to active trypsin, and thus probably cause premature, intrapancreatic trypsinogen activation disturbing the intrapancreatic balance of proteases and their inhibitors. Other genes, such as anionic trypsinogen (PRSS2), the serine protease inhibitor, Kazal type 1 (SPINK1) and the cystic fibrosis transmembrane conductance regulator (CFTR) have been found to be associated with chronic pancreatitis (idiopathic and hereditary) as well. Furthermore, a trypsin receptor of the protease-activated receptor (PAR) family, PAR-2, has been seen to influence the onset and aggravation of pancreatitis [29]. Theoretically, protease inhibitors can inhibit repetitive activation of intrapancreatic trypsin if they can arrive at their target. Camostat mesilate is an orally active protease inhibitor and its primary effect is the inhibition of trypsin. Dr. Motoo [31] reviewed the potential effects of camostat on the pathogenesis and exacerbation of chronic pancreatitis, and evaluated several effects on the pancreas, such as the suppression of inflammatory mediators, its influence on apoptosis and on regulating fibrosis. Jia et al. [32] reported the suppressive effects of camostat on the expression of IL-1beta, IL-6, TNF-alpha, TGF-beta, and alpha-SMA in spontaneous diabetic Otsuka Long-Evans Tokushima fatty (OLETF) rats. They suggested that camostat effectively inhibits inflammation and fibrosis of the pancreas by suppressing cytokines. Pancreatic stellate cells (PaSCs) are myofibroblast-like cells found in the areas of the pancreas which have an exocrine function [33]. The activation of PaSCs induces them to proliferate, to migrate to sites of tissue damage, to contact and possibly phagocytose, and to synthesize extracellular matrix (ECM) components to promote tissue repair. The sustained activation of PaSCs leads to an imbalance between extracellular matrix protein synthesis and degradation, eventually resulting in pancreatic fibrosis associated with chronic pancreatitis and with pancreatic cancer. Gibo et al. [34] demonstrated that camostat prevented the progression of pancreatic fibrosis in rats. These observations that camostat inhibited monocyte chemoattractant protein-1 (MCP-1) and TNF-alpha production by cultured monocytes, the proliferation of PaSCs and MCP-1 production by PaSCs in vitro proved the existence of the direct effect of camostat on immunocompetent cells and PaSCs. PaSCs could be another target of protease inhibitors.
Protease Inhibitors and Pancreatic Cancer

Recently, many tumor-specific alterations have been discovered and evaluated. These alterations create a tumor-specific environment with many potential targets for therapy (targeted therapy). Proteases play an important role in cancer invasion and metastasis [35]. Serine proteases and matrix metalloproteinases (MMPs) are the focus of intense research, as they appear to be related to the process of tumor progression [36]. Three classes of proteases have been associated with focal degradation of the basement membrane. These three groups include the serine proteases (such as the plasmin/plasminogen system), the cathepsin proteases, and the matrix metalloproteinases. These various proteases work both independently and in concert to advance tumor progression and metastases.

In this Round Table, Dr. Uchima et al. [37] discussed the effect of protease inhibitors on serine proteases and MMP, such as urokinase-type plasminogen activator (u-PA) and tumor associated trypsinogen (TAT), MMP-2, MMP-9 and membrane type-MMPs (MT-MMPs). Physiological serine protease inhibitors, such as urokinase-type plasminogen activator (uPA), and its inhibitor, plasminogen activator inhibitor-type 1 (PAI-1), play a key role in tumor invasion and metastasis in many cancers. On the other hand, gabexate is a well-known non-physiologic, synthetic serine protease inhibitor. Several studies from Dr. Uchima’s group [38, 39, 40] reported the inhibitory effects of gabexate on pancreatic cancer cell invasion by directly antagonizing the activities of uPA and TAT. On the other hand, Dr. Takahashi et al. [41] discussed the effects of protease inhibitors on NF-kappa B in pancreatic cancer. They demonstrated the relationship between the glial cell-line derived neurotrophic factor (GDNF) and perineural invasion by human pancreatic cancer cells, and confirmed that NF-kappa B is a part of the signaling pathway from the GDNF in human pancreatic cancer cells. GDNF increased NF-kappa B activity in human pancreatic cell lines and the invasive potential is regulated by NF-kappa B activation. They documented the inhibitory effect of gabexate for pancreatic cancer invasion. They also demonstrated that gabexate suppressed TNF-alpha-induced NF-kappa B activation and enhanced apoptosis in human pancreatic cancer cell lines [3]. Gabexate has been reported to inhibit NF-kappa B activation in human monocytes and umbilical vein endothelial cells. NF-kappa B has various functions in cancer cells, including the prevention of apoptosis and promotion of chemoresistance, cell invasion, and metastases.

Recently, another synthetic trypsin inhibitor, nafamostat mesilate (FUT-175), has been reported to disrupt interconnected signaling pathways both by suppressing the NF-kappa B antiapoptotic activity and inducing tumor necrosis factor receptor (TNFR)-mediated apoptosis [42]. They found that nafamostat inhibited NF-kappa B activation by suppressing I-kappa B kinase complex (IKK)-mediated I-kappa B-alpha phosphorylation and simultaneously induced TNFR1-mediated caspase-8 activation. Both of these effects resulted in apoptosis. Nafamostat is both a potent inhibitor of NF-kappa B activity by blocking IKK-mediated phosphorylation of I-kappa B-alpha, and a strong inducer of apoptosis by up-regulating the expression of TNFR1, thereby enhancing TNFR1-mediated apoptosis. These results suggest a possible mechanism by which nafamostat suppresses NF-kappa B antiapoptotic activity and induces TNFR-mediated apoptosis. Both gabexate and nafamostat can function as NF-kappa B inhibitors and apoptosis inducers; however, their mechanisms of actions are not fully understood. The effects of protease inhibitors may involve TNFR1-mediated signaling cascades which activate NF-kappa B and the suppression of inflammatory responses, largely attributed to its inhibitory effect on proteases. Moreover, synthetic protease inhibitors could be a potentially therapeutic agent for pancreatic cancer.

Knowledge regarding the role of proteases in
tumor progression, invasion and metastasis should be used in the development of specific inhibitors to block their molecular mechanisms of action. Continuous inhibition of the molecular mechanism of pancreatic cancer by gabexate or nafamostat should be effective, but both protease inhibitors should be administered intravenously and have a very short half-time in the blood. An orally active protease inhibitor (camostat) seems to be easy to use and has inhibitory effects on serine proteases, MMP, and NF-kappa B, modulating pancreatic cancer cell invasion or metastasis in vitro and in vivo studies.

Conclusion

Proteases have various effects on the onset and multiple cascades which independently alter the course of pancreatitis and pancreatic cancer. It does not seem to be enough to prevent a single cascade for the treatment of pancreatic diseases. Further research is needed for a multi-drug approach including protease inhibitors. In the treatment of acute pancreatitis, the timing, dosage and route of administration of a protease inhibitor are also important. Some justification exists for the use of protease inhibitors in the early stages of acute pancreatitis, but the data are insufficient to recommend it as a standard of care. Protease inhibitors could be involved in several therapeutic methods of targeting at NF-kappa B. NF-kappa B comprises a family of transcription factors which activate the expression of a wide array of genes involved in tumor-genesis, metastasis, differentiation, embryonic development, apoptosis and inflammation in pancreatitis and pancreatic cancer.

In conclusion, we believe that protease inhibitors may constitute an important and active field of study through the discussion in this virtual Round Table; therefore, we should continue to study protease inhibitors for clinical use in pancreatitis and pancreatic cancer.

Keywords

NF-kappa B; Pancreatic Neoplasms; Pancreatitis; Protease Inhibitors; Receptor, PAR-2

Abbreviations

CRAI: continuous regional arterial infusion; GDNF: glial cell-line derived neurotrophic factor; MCP: monocyte chemoattractant protein; MMP: matrix metalloproteinase; MT: membrane type; OLETF: Otsuka Long-Evans Tokushima fatty; PAF: platelet-activating factor; PAI plasminogen activator inhibitor; PAR: protease-activated receptor; PaSC: pancreatic stellate cells; TAT: tumor associated trypsinogen; TNFR: tumor necrosis factor receptor; u-PA: urokinase-type plasminogen activator

Conflict of interest

The authors have no potential conflicts of interest

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