JOP. J Pancreas (Online) 2007; 8(4 Suppl.):533-537.

ROUND TABLE

Antiproteases in the Treatment of Chronic Pancreatitis

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Introduction

Oral antiproteases or protease inhibitors are currently used in Japan in clinical settings as a medical treatment for chronic pancreatitis. However, their precise mechanisms are yet to be elucidated. Repetitive attacks of acute pancreatitis result in the development of chronic pancreatitis. Oral antiproteases are effective for these acute attacks. In this round table discussion, I would like to review the clinical and experimental data on antiproteases, especially camostat mesilate.

Clinical Aspect

In a recent study by Ito *et al*. [1], 656 patients with confirmed chronic pancreatitis were studied and followed up for 8 years as regard their development of diabetes mellitus. In 1994, 35.1% of the 656 chronic pancreatitis patients had diabetes mellitus; the incidence of diabetes mellitus went up to 50.4% in 2002. There were 418 patients without diabetes mellitus in 1994, but 28.9% (121/418) were newly diagnosed as having diabetes mellitus in 2002. Patients treated with camostat mesilate (CM) developed diabetes mellitus less frequently as compared to those who were not treated with camostat mesilate.

Ito *et al*. [2] conducted a prospective controlled study on the effectiveness of camostat mesilate for possible (clinically suspect) chronic pancreatitis. Camostat mesilate tended to attenuate the elevation of serum amylase and improve subjective complaints.

It is sometimes difficult to differentiate functional dyspepsia from chronic pancreatitis. Camostat mesilate is also reported to be effective in the treatment of functional dyspepsia [3].

The human pancreas adapts to the oral administration of antiproteases [4]. Camostat mesilate in healthy volunteers showed a hypertrophic effect on the pancreas, increased cholecystokinin (CCK) release, and changed pancreatic enzyme secretion [4].

Experimental Evidence

There are only a few experimental models for chronic pancreatitis. The WBN/Kob (Wistar-Bonn-Kobori) rat develops chronic pancreatitis at 12 weeks of age when it is fed a protein-rich, fat-rich MB-3 (Funabashi Farm, Funabashi, Japan) diet [5]. This model does not need any stresses or specific drugs in order to develop chronic pancreatitis. Oral administration of camostat mesilate to this spontaneous chronic pancreatitis model increased pancreatic wet weight at 8 weeks, suppressed mRNA expression of pancreatitis-associated protein (PAP), p8, IL-6 and TGF-beta at 12 weeks, and reduced pathological scores at 16 weeks as compared to untreated controls [5]. Increased pancreatic wet weight means decreased pancreatic atrophy caused by pancreatic acinar apoptosis. Suppression of the expression of PAP, p8 and various cytokines means that camostat mesilate inhibits local inflammatory reactions by means of the regulation of inflammatory mediators. PAP has recently been recognized as an anti-inflammatory cytokine [6].

PAP and p8 are also involved in acinar cell apoptosis, and these anti-apoptotic factors are expressed in acinar cells as a self-defense reaction in order to avoid further apoptosis. Suppression of these factors by camostat mesilate seems paradoxical, but the primary effect of camostat mesilate is the suppression of inflammation, which leads to less apoptosis, and it would no longer be necessary for the pancreas to express PAP and p8.

TGF-beta is deeply involved in pancreatic fibrosis [7]. The suppression of TGF-beta expression leads to the inhibition of pancreatic fibrosis [8]. Pathological scores include inflammatory reaction, fibrosis and acinar destruction.

We also looked at the expression of pro-apoptotic factors, such as tumor protein p53-induced nuclear protein 1 (TP53INP1), in the same experimental model. Camostat mesilate suppressed the expression of TP53INP1 at 12 weeks when the inflammatory changes are most prominent [9].

As for acinar cell apoptosis in the WBN/Kob rat, our previous reports show two peaks, at 12 and at 20 weeks, in the time course of the apoptotic index calculated using the terminal deoxynucleotidyl transferase nick end labeling (TUNEL) assay [10]. There are also two peaks at the same time points in the expression kinetics of pro-apoptotic factors such as Fas and Fas ligand [10]. On the other hand, there is only a single peak at 12 weeks in the expression of anti-apoptotic factors such as PAP [11], p8 [12], and clusterin [13]. Tomasini *et al*. [14] confirmed that cell death occurred by apoptosis in the TP53INP1-transfected cells. Therefore TP53INP1 does promote cell death, whereas TP53INP1 is not induced by apoptosis. Since TP53INP1 is a pro-apoptotic factor, two peaks of its gene expression in the WBN/Kob rat model are consistent with the previous results on pro-apoptotic factors [15]. The first peak of apoptosis would reflect acinar cell responses to stress at the onset of chronic pancreatitis. Around this time point, the histopathology of the pancreas resembles acute edematous pancreatitis, although there is already pancreatic fibrosis [16]. The second peak of apoptosis may be involved in acinar cell remodeling and regeneration. Proliferating cell nuclear antigen (PCNA) was also up-regulated at 16 and 20 weeks in this model [17]. Therefore, it may also reflect acinar cell proliferation. Both pro- and anti-apoptotic factors are simultaneously activated in acute pancreatitis [18]. Therefore, acinar cell apoptosis is considered to have several biological functions in the course of chronic pancreatitis. First, during the acute phase of pancreatitis, apoptosis would attenuate the severity of the pancreatitis, preventing the excessive release of harmful pancreatic enzymes. Second, apoptosis is a major process of acinar cell loss in the progression of chronic pancreatitis. Third, acinar cell apoptosis could be a part of the structural alteration, or remodeling, towards pancreatic regeneration in the process of inflammation and fibrosis. The second peak of acinar cell apoptosis and pro-apoptotic factors including TP53INP1 in the WBN/Kob rat pancreas could reflect acinar cell loss and acinar remodeling or regeneration during the progression of chronic pancreatitis.

Camostat mesilate, a low-molecular-weight serine protease inhibitor, increases exocrine pancreatic secretion and pancreatic weight in rats, and reduces amylase release from pancreatic acini [19, 20]. These primary action mechanisms of camostat mesilate contribute to the anti-inflammatory effect.

Then, what about the anti-fibrotic effect? Emori *et al*. [21] reported that camostat mesilate reduced pancreatic fibrotic areas, confirming the reduction of the expression of alpha-smooth muscle actin and the pancreatic content of prolyl hydroxylase in the chronic pancreatitis model induced by the administration of diethyldithiocarbamate (DDC), a superoxide dismutase (SOD) inhibitor. Further evidence was reported by Gibo *et al*. [22]. They used the experimental pancreatic fibrosis model induced by dibutyltin dichloride (DBTC), and determined the effect of camostat mesilate on isolated monocytes and pancreatic stellate cells (PSCs). Chronic pancreatitis was induced in

male Lewis rats by a single administration of 7 mg/kg DBTC; a special diet containing 1 mg/g camostat mesilate was fed to the DBTC+camostat mesilate-treated group from day 7. *In vivo*, the oral administration of camostat mesilate inhibited inflammation, cytokine expression and fibrosis in the pancreas. The *in vitro* study revealed that camostat mesilate inhibited both monocyte chemoattractant protein (MCP)-1 and tumor necrosis factor (TNF)-alpha production from monocytes, and proliferation and MCP-1 production from PSCs. Therefore, camostat mesilate attenuated DBTC-induced rat pancreatic fibrosis by means of the inhibition of monocytes and PSC activity. Nakamura *et al*. [23] also showed the anti-fibrotic effect of camostat mesilate, focusing on pancreatic periacinar fibroblast-like cells.

Sugiyama *et al*. [24] reported that the oral administration of camostat mesilate has both preventive and therapeutic effects on chronic pancreatitis lesions and pancreatic dysfunction in the WBN/Kob rat. They speculated its mechanism of action as a stimulation of endogenous cholecystokinin (CCK) release. They [25] also revealed that camostat mesilate decreased fasting gallbladder volume by means of a mechanism other than cholecystokinin release, indicating that camostat mesilate affects gallbladder motility. As Jia *et al*. [26] reported, even in CCK-1 receptor-deficient Otsuka Long-Evans Tokushima Fatty (OLETF) rats, camostat mesilate strongly inhibits pancreatic inflammation, and prevents and reverses fibrosis and atrophy of the pancreas [26].

Finally, I would like to discuss hypertrophy of the pancreas resulting from the oral administration of camostat mesilate. Camostat mesilate is reported to induce hypertrophy in the rat pancreas by means of an increase in CCK release [27, 28]. Sato *et al*. [29] studied the trophic effect of camostat mesilate in gene-targeted mice. In their experiments, the ratio of pancreatic wet weight/body weight was significantly lower in CCK-A receptor (-/-) than (+/+) mice after a 14-day camostat mesilate treatment. Camostat showed a trophic effect on the mouse and this effect is mediated by means of the CCK-A receptor, but is less potent than in rats. Guo *et al*. [30] showed that camostat mesilate induces early response gene expressions such as c-*jun* and c-*fos* and AP-1 DNA binding, and that these effects are mainly CCK-dependent. The mechanism of the trophic effect of camostat mesilate on the pancreas is yet to be elucidated.

Conclusions

The therapeutic approach to chronic pancreatitis has progressed owing to the identification of pancreatic stellate cells and better understanding of the molecular mechanisms of chronic pancreatitis. Growth factors, cytokines, chemokines, peroxisome proliferators-activated receptor-gamma (PPAR-gamma), etc. are involved in the development and progression of chronic pancreatitis [31]. Among the drugs reacting with the above factors, antiproteases or protease inhibitors such as camostat mesilate play an important role in the therapeutic strategy of chronic pancreatitis.

Keywords Apoptosis; Biomedical Research; Cholecystokinin; Cystic Fibrosis; Cytokines; pancreatitis-associated protein; Pancreatitis, Chronic; Protease Inhibitors; Therapeutics

Abbreviations DBTC: dibutyltin dichloride; MCP: monocyte chemoattractant protein; p8: p8 protein; PAP: pancreatitis-associated protein; TP53INP1: tumor protein p53-induced nuclear protein 1

Conflict of interest The author has no potential conflicts of interest

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Document URL: http://www.joplink.net/prev/200707/31.html

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