EFFECTS OF ANTIFIBROTIC SUBSTANCES ON PANCREATIC FIBROSIS FOLLOWING ACUTE NECROTIZING PANCREATITIS

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Summary

This study was designed to search for a way to inhibit pancreatic fibrosis following acute pancreatitis. Experimental necrotizing pancreatitis was induced by a freezing procedure in the pancreas of male Wistar rats. After the freezing procedure, the rats were divided into 3 groups: nothing addition was done to the control group, while the other 2 groups received daily intraperitoneal administration of antifibrotic substances (colchicine or L-azetidine-2-carboxylic acid (AZC)) for 6 weeks. Pancreatic enzymes in the serum were not markedly influenced by administration of antifibrotic substances, and there were no differences in the ratios of dry to wet weights of the pancreas between groups with and without these drugs. After freezing, the hydroxyproline levels in the pancreas of the control group increased from 1 to 4 weeks and then decreased during the 5th and 6th weeks. All groups receiving colchicine or AZC exhibited a significant decrease in the hydroxyproline levels at 2 to 4 weeks compared with the control group (P<0.01). Histological examination also showed the inhibition of pancreatic fibrosis, agreeing with changes in the hydroxyproline levels in groups receiving colchicine or AZC. These results suggest that administration of antifibrotic substances, colchicine and AZC, have the possibility of inhibiting pancreatic fibrosis following acute pancreatitis.

Key Words: Antifibrotic substances, Hydroxyproline, Necrotizing pancreatitis, Pancreatic fibrosis.

Introduction

It is generally thought that the characteristic of chronic pancreatitis\textsuperscript{1} is an irreversible and progressive fibrous proliferation in the pancreas. This fibrosis causes not only morphologic alteration but also functional impairment in the pancreas. In most patients with acute pancreatitis, the pathological changes in the pancreas normalize together with reduction of inflammation, but in 20% or 30% of cases\textsuperscript{2} the necrotized parenchyma is replaced with fibrous tissue. However, at present there is no effective treatment to reduce this pancreatic fibrosis.

Hepatic and pneumonic fibroses have been widely studied\textsuperscript{3–5}. In the former, it is thought that as a repair mechanism in tissue after successive necrosis of liver cells, fibrous proliferation occurs, fundamentally similar to wound healing. On the other hand, the latter has been studied from the standpoint of immunology. We investigated experimentally a relationship between pancreatic fibrosis and collagenolytic

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activity in pancreatic tissue following necrotizing pancreatitis. Besides such basic studies related to pancreatic fibrosis, there are a few studies concerning prevention of or therapy for fibrous proliferation in the pancreas after acute pancreatitis. In this study, 2 antifibrotic substances were administered to rats with acute pancreatitis, and their effects on pancreatic fibrosis were examined.

**Materials and Methods**

In this study 43 male Wistar rats weighing 200-250 g and fasting for one day were used. Colchicine (Wako Junyaku Co. Osaka, Japan) an anti-microtubular drug and L-azetidine-2-carboxylic acid (AZC, Sigma Chemical Co. St. Louis, U.S.A.) a proline analogue were examined concerning their effect on pancreatic fibrosis.

Experimental necrotizing pancreatitis was produced by the following procedure. The rats received intraabdominal injections (5 mg/100 g body weight) of pentobarbital sodium and underwent laparotomy. The splenic segment of the pancreas underwent a freezing procedure for 30 seconds at -60°C in a freezing apparatus (Cryos-A) using CO₂ (Fig. 1). The rats were given food ad libitum after this procedure. The rats with necrotizing pancreatitis were divided into 3 groups, consisting of a group receiving colchicine, a group receiving AZC, and a control group in which no additional procedures had been carried out. Furthermore, the colchicine group was divided into 3 subgroups. One received 0.04, another 0.2, and the third 0.4 mg/kg body weight of colchicine by injection into the abdominal cavity daily for 6 weeks from the day the pancreatitis was induced. On the other hand, the AZC group was divided into 2 subgroups, one receiving 4 and the other 20 mg/kg body weight of AZC by injection into the abdominal cavity daily for 6 weeks.

Each group was examined as follows at 1-6 weeks after the onset of acute pancreatitis. Besides main examinations, the amylase and lipase levels in the serum were determined at 1, 3 and 6 weeks after administration of colchicine or AZC. In order to determine amylase and lipase, blood was taken from the inferior vena cava. Amylase was determined by the enzymatic method (Amylase test, Wako), and lipase was done by the British anti-lase tributyrate sodium louryl sulfate-5' dithiobis (2-nitro-benzoic acid) method (BALB-BTNB, Lypase kit Dainippon Co. Osaka, Japan). In each group, a section of the pancreatic tissue, which was taken from a fixed region of the pancreas, was desiccated by freezing for 20 hours at -40°C after measurement of the wet weight, and the ratio of dry to wet weights of the pancreatic tissue was calculated. Thereafter, hydroxyproline levels in the pancreatic tissue were determined by the KISO method of Inayama et al. 7, a modification of the method of Prochop et al. All values were expressed as µg/mg dry weight of the pancreatic tissue. Furthermore, the pancreas was removed before or 1-6 weeks after the freezing procedure, and the fibrotic changes in the pancreas were evaluated histologically with hematoxylineosin and azan stains.

All data for each examination was presented