The Effects of Continuous Oral Intake of Alcohol on the Progression of Pancreatic Fibrosis in Chronic Pancreatitis Model

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The Effects of Continuous Oral Intake of Alcohol on the Progression of Pancreatic Fibrosis in Chronic Pancreatitis Model

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ABSTRACT

A rat model of chronic pancreatitis was produced by injecting solution of zein, which acts as a protein plug, and oleic acid, which is cytotoxic (ZO solution), into the pancreatic duct of rats. We examined whether the impairment of pancreatic tissue of rats after the injection of ZO solution was aggravated by free continuous oral intake of alcohol. After the injection of ZO solution into the pancreatic duct of rats, the rats were allowed to drink 20% ethanol for 28 days. The wet weight of their pancreas and their pancreatic tissues were compared to a group without alcohol intake after injection of the ZO solution. On days 14 and 28 after injection of the ZO solution into the pancreatic duct, diffuse pancreatic fibrosis was observed. In the group of rats freely drinking 20% ethanol, a slight decrease in the wet weight of the pancreas was observed, but no significant aggravation of diffuse pancreatic fibrosis was noted. In the rat injected with the ZO solution, intake of ethanol did not aggravate the progression of pancreatic fibrosis.

INTRODUCTION

The histology of chronic pancreatitis is a loss of pancreatic parenchyma and fibrosis. Because of its anatomic location, it is difficult to perform biopsies on the pancreas, and thus to examine its histologic changes. Therefore, a rat model of human chronic alcoholic pancreatitis was made by injecting zein-oleic acid (ZO) solution into the pancreatic duct. Zein is a simple protein found in corn germ milk. It is water-insoluble, and is one of the prolamines that are soluble in concentrations of ethanol of 50% or higher (TATHAM et al, 1993). This method reproducibly results in pancreatic fibrosis on day 28 after injection. Using this rat model, we examined the effects of intake of alcohol on the progression of pancreatic fibrosis in chronic pancreatitis.

MATERIALS AND METHODS

Thirty-five male Wistar rats weighing 220 to 240 g were used. The rats were purchased from Funahashi Farm, MM-3, and were housed under controlled light-dark conditions (light on 7:00-19:00) with room temperature regulated at 23-25℃. The rats were allowed free access to standard food and tap water, and were maintained and cared for humanely in accordance with the recommendations of the Guide for the care. Their body weight was measured during

the course of the experiment.

The control group consisted of seven rats which were not injected with the ZO solution, but which were allowed to drink 20% ethanol in water from a water feeding bottle (Al group). On day 28, the pancreas was removed, and its wet weight was measured.

According to our method (SHIOZAKI 1997), 37.5 mg of zein was mixed with 0.1 ml of 95% ethanol. This was then dissolved in 0.9 ml of oleic acid by slow agitation and left standing for about 24 hours, yielding a viscous solution (ZO solution). After about 16 hours of fasting, the rats were celiotomized under anesthesia induced by intra-abdominal administration of 50 mg/kg pentobarbital. The pancreatic duct was clamped at the porta hepatis, and 100 μl of ZO solution were injected into the pancreatic duct from the terminal on the duodenal side over a 2-min period using a Harvard pump. Immediately after the injection, clamping was released. After the ZO solution was confirmed to be washed out from the pancreatic duct, the abdomen was closed. On days 14 or 28, the rats (n=14) were killed, and the wet weight of the pancreas was measured (ZO group).

The 14 study rats were allowed to drink 20% ethanol in water from a feeding bottle immediately after the ZO solution injection (ZO+Al group). On days 14 or 28, the rats were killed, and the wet weight of the pancreas was measured.

Pancreatic tissues were collected from the center of the pancreaticosplenic lobe, fixed in 10% neutral formalin, stained with hematoxylin and eosin (HE) and Azan, and examined histologically.

The results are expressed as the mean±SD. Comparisons between two groups were performed using the Wilcoxon-Mann-Whitney test. Values of $p<0.05$ were considered statistically significant.

RESULTS

Changes in body weight (Fig. 1) and wet weight of the pancreas (Fig. 2)

In the Al group, the mean body weight was 333.3±7.2 g on day 28. In the ZO group, the body weight did not increase for a few days after injection but started to increase on day 4, and was 314.2±10.3 g on day 28. In the ZO+Al group, the body weight decreased, and it took 10 days for recovery. It then increased to 312.3 ±13.1 g on day 28. There was significant difference in the body weight on day 28 between Al group and ZO group, ZO+Al group ($p<0.05$). However, there was no significant difference in the body weight on day 28 between the ZO and ZO+Al groups.

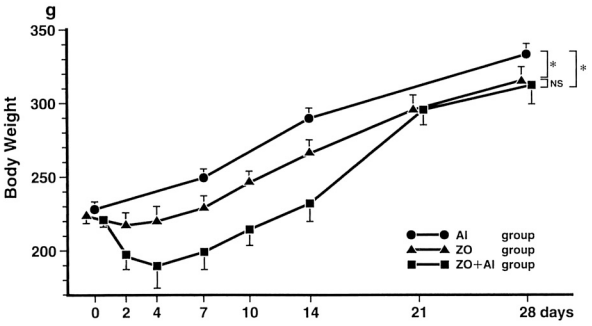

Fig. 1. Changes in the mean body weight of each experimental group until day 28
NS : not significant
*** : $p<0.05$ between groups**

The wet weight of the pancreas on day 14 was 559.2±132.1 mg in the ZO group, and 364.9 ±139.6 mg in the ZO+Al group. On the other hand, the wet weight of the pancreas on day 28 was 1201.4±40.52 mg in the Al group, 475.1±86.8 mg in the ZO group, and 376.8±73.4 mg in the ZO+Al group. There was significant differece in the wet weight of the pancreas on day 28 between Al group and ZO group, ZO+Al group ($p<0.05$). However, there was no significant difference in the wet weight of the pancreas on day 28 between the ZO and ZO+Al groups, but the ZO+Al group had the lowest value.

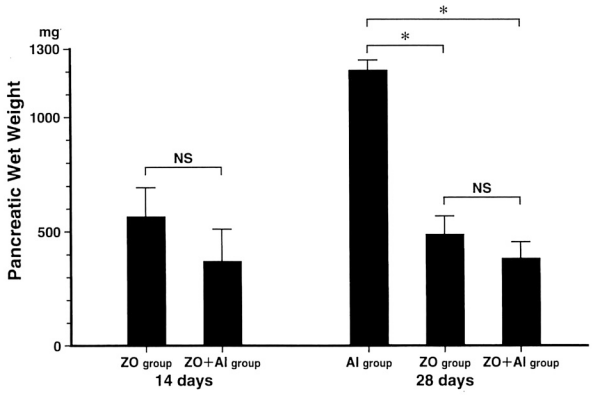

Fig. 2. Comparisons of the wet weight of the pancreas in each experimental group on days 14 and 28
NS : not significant
*** : $p<0.05$ between groups**

Histological findings of the pancreas

a) The Al group (Fig. 3)

Vacuolation was observed in some of the acinar cells in the Al group. This was most likely due to fat precipitation. No changes were observed in Langerhans' islets or epithelium of the pancreatic duct. No inflammatory cellular infiltration or fibrosis were detected.

b) The ZO group (Fig. 4)

On day 14, the acinar cells had degenerated and decreased in number, and fibrosis was noted. On day 28, the lost acinar cells were replaced with fibrotic tissue and diffuse fibrosis had progressed in the interlobular and periductal areas. Some regions, however, were replaced with fat tissues ; though the Langerhans' islets were present.

c) The ZO + Al group (Fig. 5)

On day 14, fibrosis was observed, but no regions were replaced with fat tissues. On day 28, diffuse fibrosis was observed in a manner similar to that in the ZO group. There was no evidence of histological findings to aggravate the progression of pancreatic fibrosis.

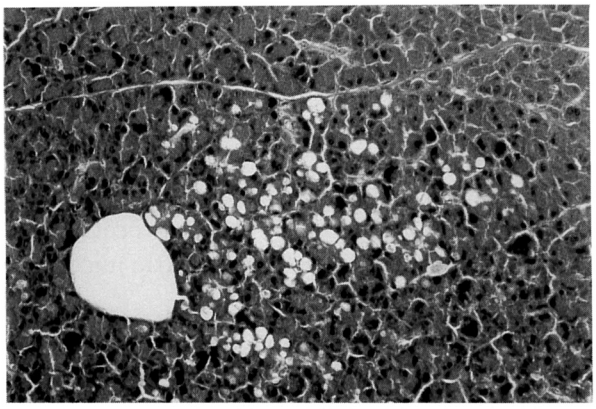

Fig. 3. Histological appearance of the pancreas 28 days after continuous intake of alcohol (A1 group, HE stain ×200)
Vacuolation was observed in some of the acinar cell. No inflammatory cellular infiltration or fibrosis were detected.

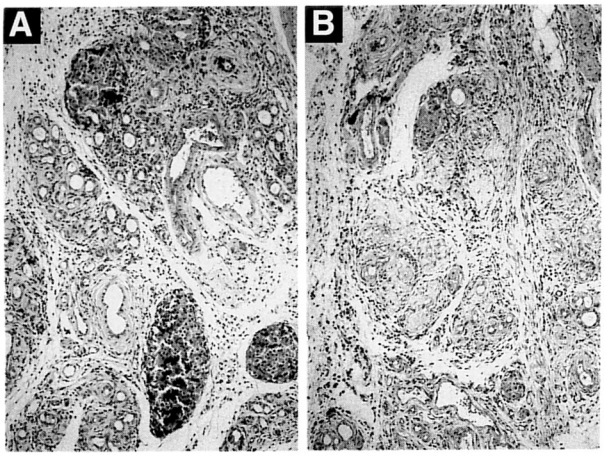

Fig. 4. Course of pancreatic histological findings after injection of ZO solution (ZO group, HE stain×100)
A : 14 days after injection
The acinar cells had degenerated and decreased in number and fibrosis was detected.
B : 28 days after injection
The lost acinar cells were replaced with fibrotic tissue and diffuse fibrosis had progressed in the interlobular and periductal areas.

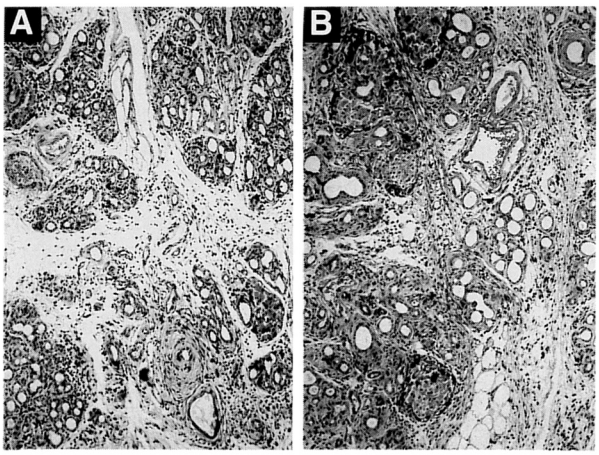

Fig. 5. Course of pancreatic histological findings after injection of ZO solution and continuous intake of alcohol. (ZO + A1 group, HE stain×100)
A : 14 days after injection.
Fibrosis was observed.
B : 28 days after injection.
Diffuse fibrosis was observed in the interlobular and periductal areas in a manner similar to that in the ZO group.

DISCUSSION

A number of attempts have been made to produce an animal model of chronic pancreatitis using long-period intake of 20% ethanol in rats. SARLES (1971) had reported that loss of acinar cells, formation of protein plugs in the pancreatic duct, and diffuse fibrosis of the stroma, similar to human chronic pancreatitis, were observed in rats with free access to alcohol for more than 1 year. While these results have not been duplicated, the hypothesis that the formation of protein plugs in the pancreatic duct is important in chronic pancreatitis has been developed into the small duct theory (SARLES 1974 ; SARLES and SARLES 1976 ; KAGAYA et al, 1979 ; SINGH et al, 1982 ; MATSUNO et al, 1983 ; PAPP et al, 1984). Therefore prolonged intake of ethanol alone does not appear to induce chronic pancreatitis in rats.

In the present study, fat precipitation were observed in the acinar cells as an early change in the pancreatic parenchyma after 1 month of oral administration of 20% ethanol. Beck et al (1974) has found almost no difference between the concentration of ethanol in arterial blood and the concentration of ethanol in pancreatic juice. This fact suggest that ethanol is not metabolized by pancreatic cells but rather enters into pancreatic juice, suggesting the possibility that ethanol directly injures the epithelium of the pancreatic duct. On the other hand, it has been shown that the pancreas has markedly high activity of fatty acid ethyl ester (FAEE) synthase in rats (LAPOSTA and LANGE, 1986). This observation implies that ethanol is metabolized without oxidation in the pancreas of rats to yield FAEE by forming an ester-bond with free fatty acid (HAMAMOTO et al, 1990). In the present study, fat deposition observed in acinar cells are thought to be a result of the increased concentration of FAEE. This supports the speculation that changes in the pancreas in the early stage after long-term administration of ethanol to rats are due to a toxic metabolic pathogenesis.

For this reason, we established a model of chronic pancreatitis exhibiting irreversible damage of acinar cells by injecting of ZO solution into the pancreatic duct. Oleic acid is cytotoxic, while zein is a simple protein that acts as a protein plug. The viscosity is increased by mixing oleic acid with zein. It should be noted that about 10% ethanol is present in the ZO solution as the solvent of zein. As a result, the ZO solution contains factors that directly cause injury to the epithelium of the pancreatic duct. This animal model may be similar to chronic alcoholic pancreatitis in humans. Two to 4 weeks after injection of the ZO solution into the pancreatic duct, progression of fibrosis was observed first in the lobes, then between the lobes, replacing the lost acinar cells. Such progression of lesions is an irreversible effect of severe inflammation in the early stage.

In the early stage after injection of ZO solution into the pancreatic duct, the peripheral pancreatic duct may be partially obstructed by zein. In this chronic pancreatitis model, impairment of acinar cells may be aggravated by free oral intake of 20% ethanol. On the other hand, exocrine pancreatic function is enhanced by secretion of secretin as well as secretion of gastric juice induced by oral intake of alcohol (CAVARZAN et al, 1975). In the early stage of chronic alcoholic pancreatitis in humans, an increase in the amount of pancreatic juice has been observed after secretin stimulation (TAKEDA et al, 1984). These findings suggest that the wash-out of zein is enhanced in the pancreatic duct in the early stage of free oral intake of ethanol, which would prevent, rather than stimulate, the progression of impairment of the pancreas. In rats, secretion of cholecystokinin (CCK) also appears to be enhanced after oral alcohol intake. In the acinar cells injured by oleic acid, enhancement of secretion by the pancreas caused by both secretin and CCK are risk factors when pancreatic juice outflow is impaired.

In the present model, free oral intake of 20% ethanol was not a factor causing progression of fibrosis of the pancreas. In our experiments, the ZO solution was injected into the pancreatic duct only once. If it had been injected several times until the 28th day of alcohol intake, alcohol may have contributed to the progression of the fibrosis of the pancreas.

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