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ORIGINAL ARTICLE

Basic Study

Effect of endogenous cholecystokinin on the course of acute pancreatitis in rats

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Abstract

AIM: To examine the effects of pancreatic rest, stimulation and rest/stimulation on the natural course of recovery after acute pancreatitis.

METHODS: Acute hemorrhagic pancreatitis (AP) was induced in male rats by intraductal infusion of 40 μL/100 g body weight of 3% sodium taurocholate. All rats took food *ad libitum*. At 24 h after induction of AP, rats were divided into four groups: control (AP-C), pancreas rest (AP-R), stimulation (AP-S), and rest/stimulation (AP-R/S). Rats in the AP-C, AP-R and AP-S groups received oral administration of 2 mL/kg body weight saline, cholecystokinin (CCK)-1 receptor antagonist, and endogenous CCK release stimulant, respectively, twice daily for 10 d, while those in the AP-R/S group received twice daily CCK-1 receptor antagonist for the first 5 d followed by twice daily CCK release stimulant for 5 d. Rats without any treatment were used as control group (Control). Biochemical and

histological changes in the pancreas, and secretory function were evaluated on day 12 at 24 h after the last treatment.

RESULTS: Feeding *ad libitum* (AP-C) delayed biochemical, histological and functional recovery from AP. In AP-C rats, bombesin-stimulated pancreatic secretory function and HOMA-β-cell score were significantly lower than those in other groups of rats. In AP-R rats, protein per DNA ratio and pancreatic exocrine secretory function were significantly low compared with those in Control rats. In AP-S and AP-R/S rats, the above parameters recovered to the Control levels. Bombesin-stimulated pancreatic exocrine response in AP-R/S rats was higher than in AP-S rats and almost returned to control levels. In the pancreas of AP-C rats, destruction of pancreatic acini, marked infiltration of inflammatory cells, and strong expression of α-smooth muscle actin, tumor necrosis factor-α and interleukin-1β were seen. Pancreatic rest reversed these histological alterations, but not atrophy of pancreatic acini and mild infiltration of inflammatory cells. In AP-S and AP-R/S rats, the pancreas showed almost normal architecture.

CONCLUSION: The favorable treatment strategy for AP is to keep the pancreas at rest during an early stage followed by pancreatic stimulation by promoting endogenous CCK release.

Key words: Acute pancreatitis; Pancreatic stimulation; Cholecystokinin; Pancreatic rest; Pancreatic function

Core tip: In experimental acute hemorrhagic pancreatitis, feeding *ad libitum* without any treatment delayed biochemical, histological and functional recovery. Both pancreatic rest made by blocking cholecystokinin (CCK)-1 receptor and pancreatic stimulation caused by eliciting endogenous CCK release improved biochemical and histological alterations, except pancreatic secretory function. The favorable treatment strategy for acute pancreatitis (AP) is to keep the pancreas at rest during an early stage followed by pancreatic stimulation. Thus, high-protein meals should be avoided during the early phase after AP but protein meals may be important at later times to stimulate recovery of pancreatic function.

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INTRODUCTION

Acute pancreatitis (AP) is an inflammatory disease occurring in the pancreas. It is assumed that inappropriately activated trypsin triggers a chain of intracellular zymogen activation in the pancreas, resulting in AP[1,2]. Regardless of the underlying causes, vigorous intravenous hydration is the first important treatment principle of AP to stabilize blood pressure and intravascular volume, and prevent hypovolemic shock[3,4]. In addition to fluid resuscitation, traditional treatment consists of initial fasting to suppress synthesis and secretion of pancreatic enzymes, and avoid activation of proteolytic enzymes[5]. Food intake would elicit endogenous release of cholecystokinin (CCK) that stimulates pancreatic enzyme synthesis and secretion[6,7], which may aggravate damage of the pancreas[8,9]. Similarly, previous studies have demonstrated that exogenous injection of cerulein or CCK-8, even at physiological doses, worsens the mortality and morbidity in AP in rats and mice[10,11]. Indeed, fasting decreased endogenous CCK concentrations and ameliorated the severity of AP[12]. In addition, we have demonstrated that CCK-1-receptor-deficient Otsuka Long-Evans Tokusima Fatty (OLETF) rats do not develop severe AP, although plasma CCK levels rise up to 4-14-fold over the preloading values after the onset of AP[13]. In concert with these observations, numerous studies have shown that potent and specific CCK-1 receptor antagonists reduce the severity of pancreatitis in animal experiments[8,9,14] and clinical trials[15,16]. These results suggest that pancreatic rest may promote healing, decrease pain, and reduce secretion and complications.

However, patients with AP maintain an accelerated basal metabolic rate, protein catabolism increases by 80% and energy expenditure by 20%, and therefore have increased caloric needs, therefore, nutritional support is especially important[17,18]. Although parenteral nutrition (PN) was traditionally used to maintain pancreatic rest by avoiding gastrointestinal (GI) hormone release and supporting nutritional needs, avoidance of using the GI tract in patients with AP exacerbated the severity of AP, leading to greater incidence of complications and prolonged hospitalization[19,20]. Enteral nutrition (EN), in comparison to PN, significantly reduces systemic infections, pancreatic infections, surgical interventions, length of hospital stay, and mortality. It is generally accepted that EN is significantly superior to total PN regarding mortality, infectious complications, and organ failure[19,20]. It is conceivable that EN may improve outcome in patients with AP if given early[21,22]. Indeed, a randomized clinical study has revealed that immediate oral feeding in patients with mild AP may accelerate recovery[21,22]. However, there is no report regarding recovery of pancreatic function by oral nutrition from an early stage after AP. Moreover, it is unknown whether early feeding in AP improves histological alterations, or pancreatic exocrine and endocrine function. It is reported that recovery to normal does not necessarily occur after AP and that progression to chronic pancreatitis is possible in a considerable number of cases[23].

In the present study, we examined pancreatic histology and function in post-pancreatitic rats after feeding with a normal rat diet, keeping the pancreas at rest by blocking CCK-1 receptor, or stimulating the pancreas by eliciting endogenous CCK release.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 230-250 g were used in all experiments. The animals were kept in a temperature- (23 ± 2 ℃) and humidity- (55% ± 5%) controlled room with a 12-h light/dark cycle (lights on at 07:00 am). The animals were provided *ad libitum* standard rat chow consisting of (as a percentage of calories) 61% carbohydrate, 26% protein, and 13% fat (3.596 kcal/g diet: Oriental Yeast, Tokyo, Japan) and tap water.

Animal care guidelines

Our institutional Animal Welfare Committee approved the experimental protocol, and rats received humane care according to the guidelines of our institution. All experiments were performed according to the guidelines of the Ethics Committee of Animal Care and Experimentation at University of Occupational and Environmental Health, Japan. Animals were kept under specific pathogen-free conditions.

Induction of AP

Pancreatitis was induced in overnight-fasted rats according to the method of Aho *et al*[24] by retrograde intraductal infusion of 40 μL/100 g body weight of 3% taurocholic acid sodium salt (NaTc) (Sigma, St. Louis, MO, United States) dissolved in saline. Intraductal infusion was performed under steady manual pressure over a period of 30 s[13]. Rats without intraductal infusion were used as untreated normal controls (Control group).

Pancreatic rest and stimulation

At 24 h after induction of acute hemorrhagic pancreatitis, rats were divided into four different treatment groups: standard rat chow (AP-C); standard rat chow with pancreatic rest (AP-R); standard rat chow with pancreatic stimulation (AP-S); and standard rat chow with pancreatic rest, followed by pancreatic stimulation (AP-R/S). Rats in the AP-C group received 2 mL/kg body weight saline orally (po) *via* an orogastric tube twice daily (09:00 and 21:00 h) for 10 d; the AP-R group received 50 mg/kg body weight of CCK-1 receptor antagonist loxiglumide[25] (kindly supplied by Kaken Pharmaceutical Co., Tokyo, Japan) dissolved in 2 mL distilled water po twice daily for 10 d; the AP-S group received 25 mg/kg body weight protease inhibitor camostat (a generous gift from Ono Pharmaceutical Co., Osaka, Japan), which is known to stimulate endogenous CCK release[26-28], dissolved in 2 mL distilled water po twice daily for 10 d; and the AP-R/S group received 50 mg/kg body weight loxiglumide twice daily for the first 5 d followed by 25 mg/kg body weight camostat twice daily for the next 5 d. Rats were fed *ad libitum.* On day 12 at 24 h after the last treatment and overnight fasting, pancreatic exocrine function and histological examination of the pancreas were performed.

Based on our previous studies, we used CCK-1 receptor antagonist loxiglumide to make the pancreas rest[25] and synthetic protease inhibitor camostat to stimulate the pancreas *via* endogenous CCK release (pancreas stimulation)[26-28].

Exocrine secretory function

Rats were weighed before the experiment, and anesthesia was induced by subcutaneous (sc) injection of sodium pentobarbital (50 mg/kg body weight) after an overnight fast. After collecting blood for measurement of serum concentrations of glucose and insulin, the left jugular vein, and bile and pancreatic ducts were cannulated, and the pylorus was ligated. The bile was returned into the duodenum during the experiment. Pancreatic fluid secretion was obtained by replacing a calibrated tube attached to the free end of the pancreatic cannula every 10 min, and the volume and protein concentrations were determined[26,27,29,30]. The abdominal wound was covered with a saline-moistened gauze, and body temperature was maintained between 37 and 38 ℃ with a heating pad throughout the experiment. After collection of basal fluid flow, bombesin (Protein Research Institutes, Osaka, Japan) was infused into the jugular vein at a dose of 5 nmol/kg body weight/h using a syringe pump (Razal Scientific Instruments, Stanford, CT, United States) for 1 h at a rate of 1 mL/h. Pancreatic fluid was collected every 10 min.

Since there is a possibility that CCK-1 receptor antagonist loxiglumide accumulates and modifies CCK-mediated pancreatic fluid and protein secretion[29,30], and since camostat stimulates endogenous CCK release and downregulates CCK receptor[28], we used bombesin stimulation to determine pancreatic exocrine function. CCK-1 receptor antagonist is known not to inhibit the action of bombesin on rat pancreatic secretion[31].

Endocrine function and insulin resistance

Insulin secretion was calculated by homeostasis model assessment insulin secretion (HOMA-β-cell) with the following formula: fasting insulin (μU/mL) × 20/[fasting glucose (mmol/L) - 3.5], as described by Matthews *et al*[32]. HOMA-β-cell function positively correlates with the ratio of change in insulin and glucose. Insulin resistance was calculated by homeostasis model assessment insulin resistance (HOMA-IR) with the following formula: fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5, as described by Matthews *et al*[32]. With such a method, high HOMA-IR score denotes low insulin sensitivity (insulin resistance).

Measurement of pancreatic contents

After the last collection of pancreatic fluid, rats were killed, and the pancreas was excised and weighed after being trimmed free of fat, mesentery, and lymph nodes. Portions of each pancreatic tissue with similar anatomic orientation were used for histologic examination. Pieces of pancreatic tissue (100-200 mg wet weight) were homogenized in saline using a motor-driven Teflon-coated glass homogenizer at 3000 rpm (eight passes). The homogenates were filtered through three layers of gauze and then sonicated for 1 min for measurement of pancreatic contents of protein, DNA, amylase, lipase and insulin.

Histological examination

A portion of the pancreatic tissue was fixed overnight in 10% formaldehyde solution for hematoxylin and eosin (HE) staining, immunostaining, and light microscopic examination. The pathologist, without awareness of the treatment, examined all histologic samples in a single-blind fashion.

Immunohistochemistry

Paraffin-embedded pancreatic tissue sections were prepared on glass slides. Sections for interleukin (IL)-1β were pretreated in microwaves in citrate buffer (pH 6.0) for 12 min, while sections for tumor necrosis factor (TNF)-α immunohistochemistry were incubated in protease K for 10 min (for antigen retrieval). The sections for α-smooth muscle actin (SMA) immunohistochemistry were used without pretreatment. These sections were treated with graded alcohol solutions and incubated for 15 min in 3% H_2O_2 to block endogenous peroxidase activities. Nonspecific staining was blocked by incubating with bovine serum for 10 min at room temperature. Each section was incubated with goat anti-mouse TNF-α antibody (Santa Cruz Biotechnology, Santa Cruz, CA, United States) and rabbit anti-human IL-1β antibody (Santa Cruz Biotechnology) at a dilution of 1:10 at room temperature for 1 h. The sections for α-SMA immunohistochemistry were incubated with mouse anti-human α-SMA antibody (Dako Corporation, Carpinteria, CA, United States) at a dilution of 1:50 at room temperature for 30 min. In TNF-α immunohistochemistry, bound antibody was detected with rabbit anti-goat antibody (Dako Corporation) in dilution 1:400. In IL-1β or α-SMA immunohistochemistry, bound antibodies were detected with the peroxidase-labeled streptavidin-biotin method (LSAB Kit; Dako Corporation). Then, these sections were stained with diaminobenzidine (DAB). Counterstaining was performed with Mayer's hematoxylin, and the sections were mounted.

Assays

Serum glucose concentrations were determined by the glucose-oxidase method using a glucose kit (Glucose-E reagent; International Reagents, Kobe, Japan)[33]. Insulin concentrations in the serum and pancreatic homogenates were measured by radioimmunoassay using the double-antibody method[34] with a commercially available radioimmunoassay kit (ShionoRIA; Shionogi Pharmaceutical, Osaka, Japan) using crystalline rat insulin as a reference standard. The protein concentration in pancreatic homogenates and pancreatic fluid was determined by the method of Lowry *et al*[35]. DNA content was measured using the fluorescent dye H-33258 (Hoechst AG, Germany) according to Labarca *et al*[36]. Amylase activity in pancreatic homogenates was determined by a chromogenic method with blue-dyed starch polymer[37] and expressed in Somogyi units (SU). Lipase activity was determined according to the method of Whitaker[38] and expressed in international units (IU).

Statistical analysis

Results are expressed as the mean ± SE of at least six rats per group. Data were analyzed with the use of analysis of variance followed by Tukey's test using commercial software StatView (Abacus Concepts/Brain Power, Berkeley, CA, United States). $P < 0.05$ was considered to be statistically significant.

RESULTS

Pancreatic wet weight and protein and DNA content

In AP-C rats on day 12 after induction of AP, pancreatic wet weight and pancreatic content of protein and DNA were significantly lower than those in the Control rats (Table 1). However, protein per DNA ratio (an indicator of cell size) in AP-C rats was similar to that in the Control rats. Pancreatic rest for 10 d (AP-R) significantly increased pancreatic wet weight, and contents of protein and DNA compared with those in AP-C rats, but were significantly lower than those in Control rats. In addition, protein per DNA ratio in AP-R rats was significantly low compared with that in AP-C, AP-S and AP-R/S rats. In AP-S and AP-R/S rats, pancreatic wet weight and pancreatic contents of protein and DNA were recovered to the levels in Control rats (Table 1). There were no differences in pancreatic wet weight, and contents of protein and DNA between AP-S and AP-R/S rats. Protein per DNA ratio in AP-R/S rats was similar to that in Control rats, whereas that in AP-S rats tended to be higher than Control or AP-R/S rats, although the difference was not statistically significant (Table 1).

Exocrine function in response to bombesin stimulation

Basal pancreatic fluid secretion in AP-C rats was not significantly different from that in the Control rats, whereas it was significantly increased in AP-R, AP-S and AP-R/S rats compared with that in AP-C or Control rats (Figure 1A). Basal protein output in AP-C rats was significantly lower than that in Control, AP-S and AP-R/S rats (Figure 1B). Pancreatic fluid secretion and

Table 1 Effect of pancreatic rest or stimulation on the recovery of the pancreas after acute pancreatitis

Parameters	Control	AP-C	AP-R	AP-S	AP-R/S
Pancreatic wet weight (mg/rat)	1120 ± 30	698 ± 58[1]	881 ± 37[1,2]	1074 ± 69[2,3]	1079 ± 66[2,3]
Pancreatic contents					
Protein (mg/pancreas)	132 ± 8	50 ± 9[1]	89 ± 4[1]	131 ± 18[2,3]	126 ± 13[2,3]
DNA (mg/pancreas)	4.2 ± 0.2	1.7 ± 0.3[1]	3.3 ± 0.1[1,2]	3.5 ± 0.4[2]	4.1 ± 0.4[2]
Protein/DNA (mg/mg)	31.1 ± 0.8	31.3 ± 2.2	27.2 ± 1.3[1,2]	37.7 ± 1.4[3]	31.3 ± 1.3[3]
Amylase					
(103 SU/pancreas)	91.2 ± 17.7	12.3 ± 5.2[1]	46.7 ± 7.8[1,2]	67.7 ± 13.4[2,3]	73.4 ± 12.7[2,3]
(SU/mg protein)	681 ± 105	240 ± 49[1]	520 ± 96[2]	502 ± 72[2]	509 ± 78[2]
(103 SU/mg DNA)	20.4 ± 3	7.0 ± 1.2[1]	14.2 ± 3.2[2]	20.0 ± 3.7[2]	17.6 ± 2.5[2]
Lipase					
(103 U/pancreas)	11.6 ± 1.2	4.5 ± 1.2[1]	8.0 ± 0.6[1,2]	11.4 ± 1.9[2]	12.4 ± 1.6[2]
(U/mg protein)	123.3 ± 22.7	77.4 ± 8.0[1]	86.3 ± 5.9[1]	91.7 ± 6.3[2]	97.9 ± 7.1[2]
(103 U/mg DNA)	3.7 ± 0.6	2.6 ± 0.4[1]	2.7 ± 0.1[1]	3.4 ± 0.3[2]	3.1 ± 0.3[2]
Insulin content					
(nmol/pancreas)	20.2 ± 1.2	12.8 ± 0.8[1]	18.9 ± 1.5[2]	16.8 ± 0.6[2]	17.2 ± 0.5[2]
(nmol/mg DNA)	4.95 ± 0.22	8.56 ± 1.10[1]	5.56 ± 0.57[2]	5.25 ± 1.00[2]	4.80 ± 0.55[2]

[1]Significant difference *vs* control; [2]Significant difference *vs* AP-C; [3]Significant difference *vs* AP-R. Values are the mean ± SE of 6-8 rats. At 24 h after induction of acute hemorrhagic pancreatitis by retrograde intraductal infusion of 40 μL/100 g body weight of 3% NaTc, rats were divided into four groups. Rats in the AP-C group received 2 mL/kg body weight saline twice daily for 10 d; the AP-R group received 50 mg/kg body weight CCK receptor antagonist loxiglumide twice daily for 10 d; the AP-S group received 25 mg/kg body weight protease inhibitor camostat twice daily for 10 d; and the AP-R/S group received 50 mg/kg body weight loxiglumide twice daily for the first 5 d followed by 25 mg/kg body weight camostat twice daily for the next 5 d. Rats were fed *ad libitum*. On day 12 at 24 h after the last treatment and an overnight fasting, pancreatic exocrine function and histological examination of the pancreas were performed.

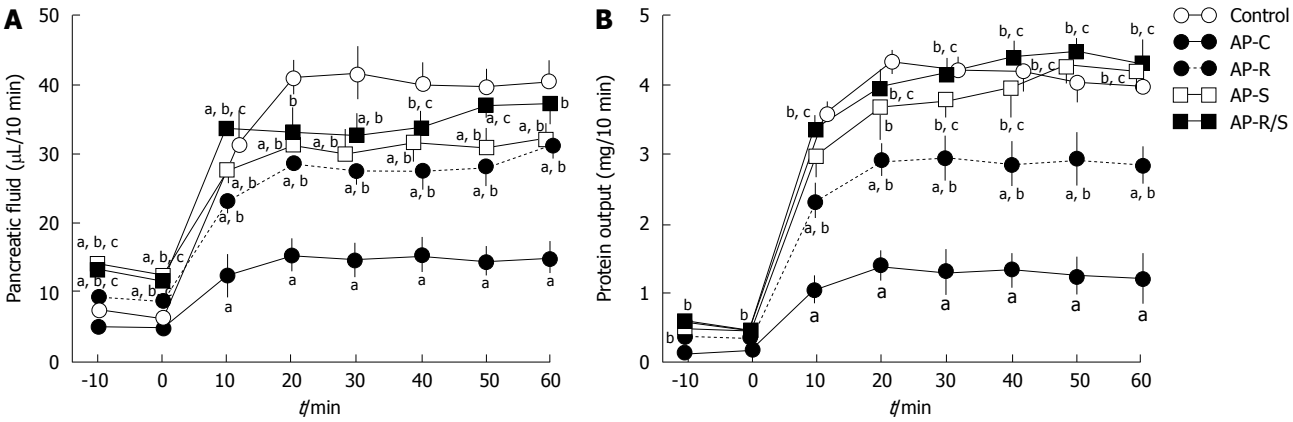

Figure 1 Pancreatic fluid secretion (A) and protein output (B) in response to bombesin stimulation in the four different treatment groups on day 12 after induction of acute pancreatitis. Results are the mean ± SE of 6-8 experiments. [a]$P < 0.05$ *vs* control; [b]$P < 0.01$ *vs* AP-C; [c]$P < 0.05$ *vs* AP-R. AP: Acute pancreatitis.

protein output in AP-C rats in response to bombesin infusion were significantly lower than those in other groups of rats (Control, AP-R, AP-S and AP-R/S) (Figure 1A and B). In AP-R rats, bombesin-stimulated pancreatic fluid secretion and protein output were significantly higher than those in AP-C rats but lower than other treatment groups (AP-S and AP-R/S) and Control. Pancreatic fluid secretion during bombesin infusion in AP-S and AP-R/S was almost similar to those in Control rats (Figure 1A and B).

Endocrine function and insulin resistance

HOMA-β-cell score (an indicator of β-cell function) in AP-C rats on day 12 after induction of pancreatitis was significantly low compared with that in the Control and AP-R, AP-R and AP-R/S groups (Figure 2A). HOMA-β-cell score in AP-R, AP-S and AP-R/S rats was similar to that in Control rats (Figure 2A). In contrast, HOMA-IR score (an indicator of insulin resistance) in AP-R rats was significantly high compared with other groups of rats, whereas that in AP-C, AP-S and AP-R/S rats was similar to that in the Control rats (Figure 2B). In AP-R/S rats, HOMA-IR score tended to be higher than Control rats, although the difference was not statistically significant (Figure 2B).

Pancreatic contents of amylase, lipase and insulin

In AP-C rats, total pancreatic contents of amylase and lipase, and concentrations of these enzymes relative to protein or DNA were lower than those in Control, AP-R, AP-S and AP-R/S rats. In AP-R rats, total amylase content was significantly lower than that

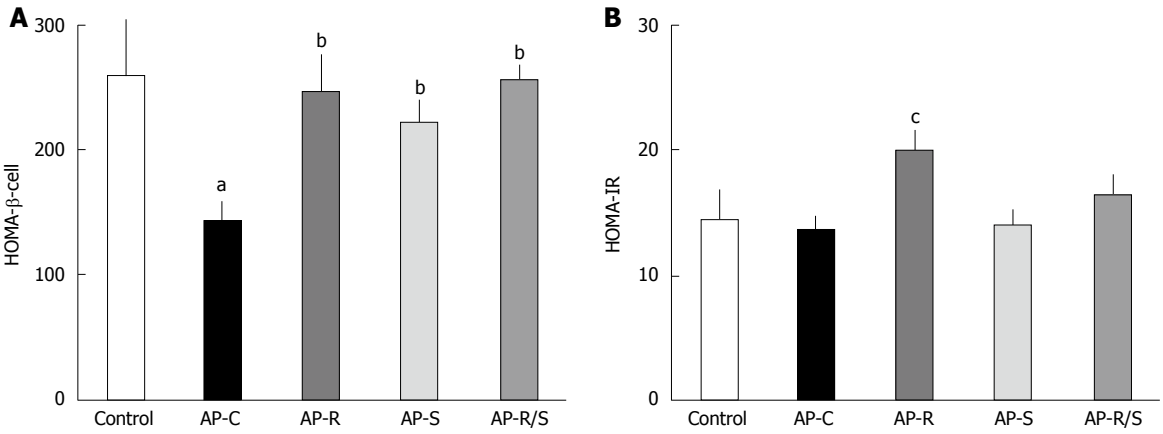

Figure 2 HOMA-β-cell (A) and HOMA-IR (B) in the four different treatment groups on day 12 after induction of acute pancreatitis. Results are the mean ± SE of 6-8 experiments. [a]*P* < 0.05 *vs* control, AP-R, AP-S and AP-R/S; [b]*P* < 0.01 *vs* AP-C; [c]*P* < 0.05 *vs* control, AP-C, AP-S and AP-R/S. AP: Acute pancreatitis.

in the Control, AP-S and AP-R/S rats (Table 1). Total pancreatic amylase content and concentrations relative to protein or DNA in AP-S and AP-R/S rats were similar to those in the Control rats. Pancreatic insulin content in AP-C rats on day 12 after induction of AP was significantly lower than that in the Control, AP-R, AP-S and AP-R/S rats. However, insulin concentration relative to DNA in AP-C rats was significantly higher than that in other groups due to a decrease in DNA content. Pancreatic rest (AP-R) or stimulation (AP-S and AP-R/S) significantly increased pancreatic insulin content to that in the Control rats (Table 1).

Histological changes

Representative photomicrographs of randomly selected sections of the pancreas taken on day 12 after induction of AP in four different treatment groups are shown in Figure 3 using the same magnification. In the pancreas of AP-C rats, destruction of pancreatic acini, tubular complexes and marked infiltration of inflammatory cells, mainly lymphocytes, were seen (Figure 3A). Pancreas rest (AP-R) for 10 d greatly reversed these histological alterations (Figure 3B), but atrophy of pancreatic acini and mild infiltration of inflammatory cells were still observed in the pancreas. These histological findings in AP-R rats were consistent with the low value of protein per DNA ratio (an indicator of cell size) compared with other groups of rats (Table 1). In AP-S (Figure 3C) and AP-R/S (Figure 3D) rats, the pancreas showed almost normal architecture.

Expression of α-SMA and cytokines

Immunohistochemical studies of pancreatic tissues of AP-C rats showed strong expression of α-SMA in the degenerative regions (Figure 4A). In AP-R rats (Figure 4D), α-SMA expression was markedly suppressed compared with AP-C rats. In AP-S (Figure 4G) and AP-R/S (Figure 4J) rats, α-SMA was only detected in pancreatic ducts. TNF-α and IL-1β were strongly expressed in inflammatory cells in the pancreas of AP-C rats (Figure 4B and C), while they were not detected in the pancreas of AP-R (Figure 4E and F), AP-S (Figure 4H and I) and AP-R/S rats (Figure 4K and L).

DISCUSSION

Food intake elicits GI hormone release such as secretin and CCK[6,7], which may stimulate the damaged pancreas in post-pancreatic states and further aggravate pancreatic inflammation. In particular, it is reported that the increased secretion of CCK is involved in aggravation of AP following administration of trypsin inhibitor[39]. Thus the principle of the traditional treatment for AP is to rest the pancreas by giving the patient nothing po but parenterally[5]. However, no advantages of PN were reported on the total hospital stay or incidences of complications of pancreatitis[19]. Absence of food in the intestine may cause intestinal atrophy resulting in bacterial translocation and multiple organ failure (MOF)[20]. Nowadays, it is generally accepted that total oral abstinence from food with PN is not beneficial to patients with severe AP, but may in fact be harmful. On the other hand, EN maintains the gut barrier, with consequent decreased bacterial translocation, which is in turn associated with fewer septic complications, and reduced surgical procedures and length of hospital stay[20]. EN within 48 h of admission was feasible and improved the clinical outcomes in mild as well as in predicted severe or severe AP by reducing complications[18]. In mild AP, immediate oral feeding is feasible and safe, and may accelerate recovery without adverse GI events[21,22]. Meta-analysis of observational data from 165 individuals from 8 randomized trials revealed that EN started within 24 h of admission reduced complications compared to EN started after 24 h of admission[21]. However, there is no report regarding recovery of pancreatic function by oral nutrition from an early time point after AP. Also, it is unknown whether after an initial attack of AP, the inflamed gland heals completely, or whether the disease progresses to chronic pancreatitis[23,40]. It is reported

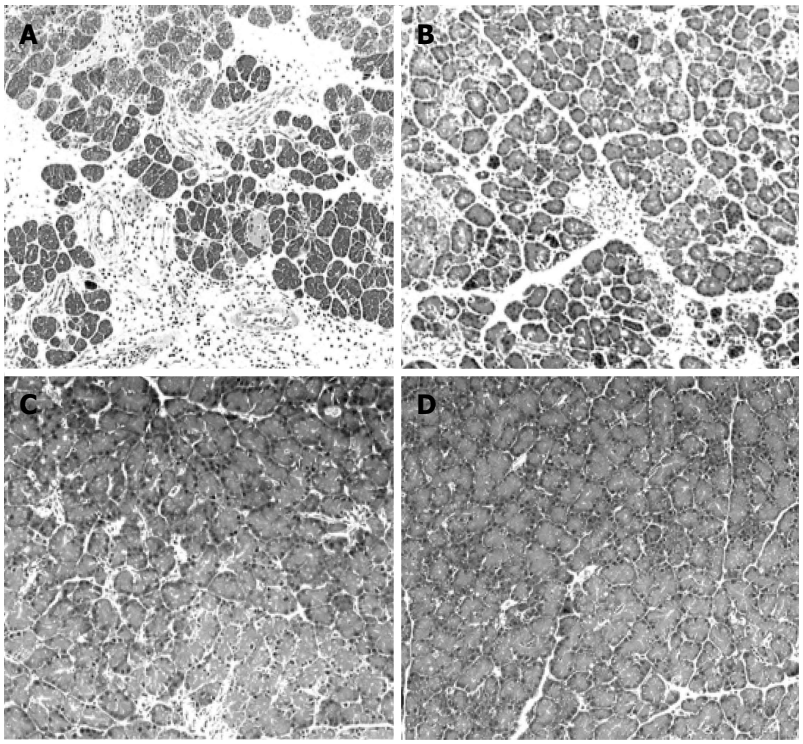

Figure 3 Representative photomicrographs of the pancreas in the four different treatment groups on day 12 after induction of acute pancreatitis. A: The pancreas of AP-C rat (*ad libitum* feeding with saline administration) showed destruction of pancreatic acini, tubular complexes and marked infiltration of inflammatory cells, mainly lymphocytes; B: The pancreas of AP-R rat (pancreatic rest) showed minimal histologic alterations with atrophic pancreatic acini and mild inflammatory cell infiltration; The pancreas in AP-S (C) (pancreatic stimulation) and AP-R/S rats (D) (pancreatic rest for the first 5 d followed by pancreatic stimulation for 5 d) showed almost normal architecture. Original magnification × 25. AP: Acute pancreatitis.

that recovery to normal does not necessarily occur after AP and that progression to chronic pancreatitis is possible at a considerable percentage[40].

In the present study, we investigated the effects of pancreatic rest by oral administration of CCK-1 receptor antagonist loxiglumide[25] and pancreas stimulation *via* endogenous CCK release induced by po protease inhibitor camostat[26-28] on the recovery of pancreatic secretory function, and biochemical and histological changes of the pancreas after acute hemorrhagic pancreatitis. Oral administration of CCK-1 receptor antagonist loxiglumide with a dose of 50 mg/kg body weight inhibited pancreatic exocrine secretion for more than 12 h[29]. Thus, every 12-h administration of loxiglumide might have completely blocked the effect of endogenously released CCK on the pancreas (pancreatic rest). On the other hand, basal plasma CCK concentrations in randomly fed rats were 2.59 ± 0.13 pmol/L, and increased to the peak of 27.6 ± 4.1 pmol/L 1 h after an oral administration of 20 mg/kg body weight camostat[27], and plasma CCK concentrations at 24 h after oral administration of 100 mg/kg body weight camostat were 6.57 ± 0.67 pmol/l, further increased to 14.24 ± 1.63 pmol/L after consecutive camostat administration for 10 d[28]. Our previous studies clearly indicate that po camostat is a strong stimulant for endogenous CCK release.

In AP-C rats that were provided *ad libitum* standard rat chow consisting of (as a percentage of calories) 61% carbohydrate, 26% protein and 13% fat with no other treatment, biochemical, histological and functional recovery from AP was delayed and incomplete, even 12 d after the attack of AP compared with that in AP-R, AP-S and AP-R/S rats. Although a previous study has revealed that EN downregulates splanchnic cytokine production and modulates the acute phase response[18], pancreatic histology and immunohistochemistry in AP-C rats suggested the presence of continued inflammatory changes in the pancreas even on day 12 after induction of AP. We found that plasma CCK levels in NaTc-induced pancreatitic rats increased from 1.6 ± 0.2 pmol/L to 22.9 ± 2.4 pmol/L at 12 h after intraductal infusion of NaTc, and remained elevated levels of 11.0 ± 1.0 pmol/L even 24 h after[13]. However, the sensitivity and responsiveness of the pancreas to CCK stimulation are decreased at an early stage of AP[41,42], therefore, the injured pancreas after AP does not respond to CCK stimulation. Thus, it is difficult to believe that the increase in plasma CCK concentrations after AP leads to exacerbation of AP. Moreover, physiological increases in plasma CCK concentrations after *ad libitum* feeding in post-pancreatitic rats seem to be too low to stimulate proliferation and growth of the damaged pancreas, and thus the recovery in AP-C rats might be delayed.

High plasma CCK concentrations are reported in patients with AP[43] as well as in various animal models of AP[8,13]. However, it is not clear whether the increase in plasma CCK concentrations is the result

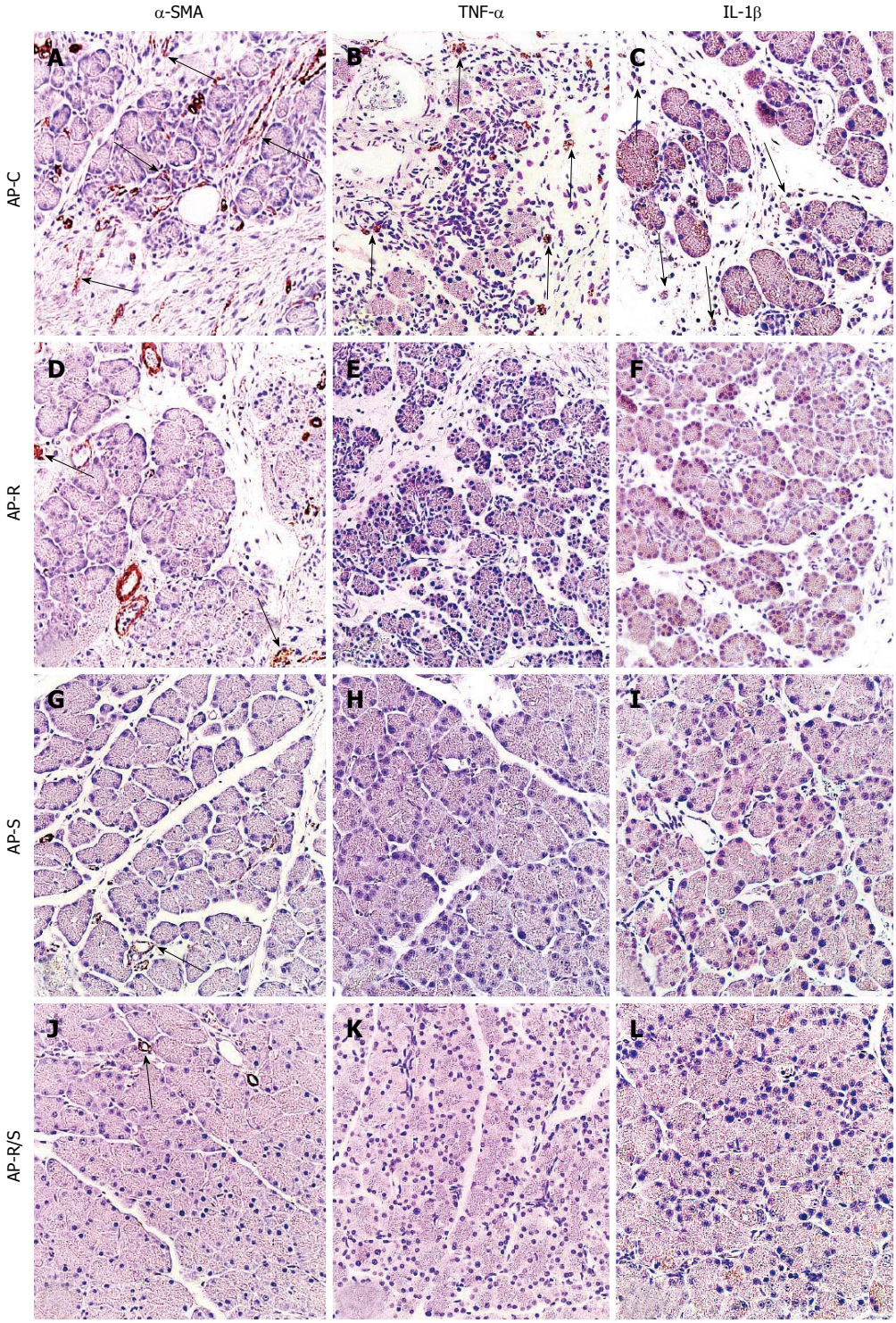

Figure 4 Representative immunohistochemical studies of the pancreas in the four different treatment groups on day 12 after induction of AP. A: The pancreas of AP-C rats showed strong α-SMA expression in the degenerative regions; In AP-S (G) and AP-R/S rats (J), α-SMA was only detected in pancreatic ducts. In AP-C rats, TNF-α (B) and IL-1β (C) were strongly expressed in inflammatory cells in the pancreas. In AP-R, AP-S and AP-R/S rats, TNF-α (E, H and K) and IL-1β (F, I and L) were not detected in the pancreas; D: Pancreatic rest (AP-R) markedly reduced α-SMA expression. Arrows in A, D, G and J indicate α-SMA expression in the pancreas. Arrows in B indicate TNF-α expression in the pancreas. Arrows in C indicate IL-1β expression in the pancreas. Original magnification × 50.

or cause of AP. Administration of excessive doses of CCK or its analog cerulein causes AP[44,45], and even physiological doses worsen AP[10]. On the other hand, CCK-1 receptor antagonists have not only preventive but also protective effects on experimental models of AP[8-11,14]. Moreover, in the OLETF rats that are missing the CCK-1 receptor, in spite of significant increase in plasma CCK concentrations after AP, biochemical, histological and functional changes are mild compared with those in the control Long-Evans Tokushima

Otsuka (LETO) rats[13]. Consistent with these reports that suggest involvement of CCK in the progress of AP, the present study demonstrated that blockade of the CCK stimulation (AP-R) accelerated the recovery compared to that in saline-treated AP-C rats. However, biochemical, histological and functional recovery in this group was still incomplete compared to that in AP-S and AP-R/S groups. Moreover, consecutive blockade of the CCK-1 receptors for 10 d appeared to delay biochemical, histological and functional recovery, since these parameters were low compared with the untreated Control rats. In addition, pancreatic rest for 10 d caused atrophy of pancreatic acini evaluated by a decrease in protein per DNA ratio, although it suppressed α-SMA and cytokines expression. Since the recovery of the injured pancreas in AP-R rats was faster than that in AP-C rats, but delayed compared with AP-S and AP-R/S rats, the regeneration process of the damaged pancreas might be under the influence of endogenous CCK at some point after AP, as previously reported[46,47]. It is conceivable therefore that the pancreatic rest should be limited only at early time points to accelerate the regeneration process of the damaged pancreas after AP. However, our previous study in cerulein-induced mild AP revealed that loxiglumide, even when given only for 3 d after the onset of AP, suppressed the spontaneous recovery of pancreatic weight and protein content evaluated on day 8[46]. The difference between the present and the previous observation might be due to the difference in severity of AP (hemorrhagic *vs* edematous pancreatitis) or to the magnitude of the elevation of endogenous CCK after AP. These differences suggest that pancreatic rest is not necessary after mild AP.

Endogenously released as well as exogenously administered CCK causes hyperplasia and hypertrophy of the pancreas, and increases pancreatic enzyme content in normal rats[26,46]. Similarly, in cerulein-induced post-pancreatitic rats, repeated sc injections of cerulein increased all parameters within 5 d and induced pancreatic growth thereafter when given after 3 d of rest[47]. Moreover, Evander *et al*[10,39] and Jurkowska *et al*[47] also demonstrated in NaTc-induced post-pancreatitic rats that soybean trypsin inhibitor (SBTI) restores the pancreas to normal after 10 d with cellular hypertrophy when started after 3 d of rest. In contrast, repeated sc injections of CCK-8 for 6 d started from 24 h after induction of cerulein-pancreatitis suppressed the spontaneous recovery of pancreatic wet weight[46]. These different results can be explained by the differences in the start of cerulein/CCK-8 or trypsin inhibitor administration after induction of AP. Both cerulein- and NaTc-induced AP rats are resistant to cerulein/CCK stimulation during an early stage of pancreatitis[41,42]. It is possible, therefore, that the repeated sc injections of CCK or endogenous CCK release during early post-acute pancreatitis (≤ 3 d after onset of AP) might have no influence on the pancreas due to cerulein/CCK resistance, and thus delayed the expected hypertrophic and hyperplastic response of the pancreas. After 3 d of rest, the post-AP pancreas might respond to CCK stimulation with hypertrophy and hyperplasia. In the present study, we used the synthetic trypsin inhibitor camostat as a stimulant for endogenous CCK release. Orally administered camostat not only elicits endogenous CCK release by inhibiting trypsin activity in the intestine, but also inhibits circulating proteases such as trypsin, kallikrein, thrombin, plasmin, and Ci esterase after being absorbed from the intestine[48]. Stimulation of the pancreas by endogenous CCK from an early time after induction of AP (AP-S) markedly decreased the expression of α-SMA and cytokines, as well as infiltration of inflammatory cells in the pancreas, and almost completely recovered pancreatic wet weight, pancreatic contents of protein, DNA and enzymes, and histology. Since the protein per DNA ratio tended to increase compared with that in Control rats, endogenously released CCK might have induced hypertrophy of the pancreas. However, pancreatic fluid secretion to bombesin stimulation was significantly low compared with that in Control rats. These results suggest that stimulation of the pancreas from an early stage after induction of AP also slightly delays the recovery.

In normal rats and mice, po administration of camostat induces pancreatic hypertrophy and hyperplasia[28,49]. However, oral administration of camostat for 10 d from an early stage after induction of AP had no significant trophic effects on the pancreas compared with untreated Control rats, although it increased pancreatic weight, and protein and enzyme contents compared with those in the AP-C rats. A similar result was observed in postpancreatitic rats injected with CCK-8 from the early stage after induction of cerulein-pancreatitis[46]. Since po administration of protease inhibitor stimulates endogenous CCK release[26-28], and endogenous CCK is shown to be an exacerbatory factor in AP[8,9,10,39], it is possible that camostat exerted an anti-protease effect but had neither hypertrophic nor hyperplasic effects on the damaged pancreas during the early stage (CCK resistant stage[41,42] after induction of AP. In contrast, however, Song *et al*[50] demonstrated a trophic effect of endogenous CCK by feeding with 0.1% camostat-containing diet for 7 d on pancreatic regeneration in severe model of acute hemorrhagic pancreatitis that was induced in rats by two intraperitoneal injections of cerulein under water-immersion stress for 5 h, once a day for three successive days.

Endogenously released as well as exogenously administered CCK plays an important role in the growth of the normal pancreas[51] and pancreatic regeneration in postpancreatitic rats[46,50]. We investigated the effect of pancreatic rest by CCK-1 antagonist for the first 5 d followed by stimulation by endogenous CCK for the next

5 d after induction of acute hemorrhagic pancreatitis (AP-R/S). By this treatment, biochemical parameters, and pancreatic endocrine and exocrine functions were completely recovered to normal. Moreover, the pancreas showed almost normal architecture and α-SMA and cytokines expression was completely inhibited. Thus, it is clear that the combination of CCK-1 receptor antagonist with an endogenous CCK release stimulant further accelerates recovery from acute hemorrhagic pancreatitis.

Pancreatitis is a complex syndrome consisting of exocrine and endocrine derangement. In the present study, we found that not only exocrine pancreas but also endocrine pancreas evaluated by HOMA-β-cell score and pancreatic insulin content were deranged in NaTc-induced AP rats. Pancreatic rest by CCK-1 receptor antagonist, and pancreatic stimulation by endogenous CCK release stimulant significantly recovered pancreatic insulin content. Thus pancreatic rest or stimulation after AP appears to improve pancreatic endocrine function, although there is a possibility that long-term CCK-1 receptor antagonist treatment causes insulin resistance as our previous reports[52,53].

In summary, long-term pancreas rest, or pancreatic stimulation from an early stage after induction of AP seems to delay the recovery. The most favorable strategy for the treatment of acute hemorrhagic pancreatitis is to maintain the pancreas at rest during an early stage for only a short period, followed by pancreatic stimulation. Although it is difficult to translate the present observation made in a particular animal model to humans, these results suggest that a high-protein meal should be avoided during an early time after AP but protein meals may be important during later times to stimulate recovery of pancreatic function.

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COMMENTS

Background

Acute pancreatitis (AP) is an inflammatory disease occurring in the pancreas. Regardless of the underlying causes, intravenous hydration, pancreatic rest, and nutritional support are important. Enteral nutrition, compared to parenteral nutrition, significantly reduces complications and mortality. However, to date, there is no report regarding recovery of pancreatic function by oral nutrition at early times after AP. Moreover, it is unknown whether early feeding in AP improves histological alterations, and pancreatic exocrine and endocrine function.

Research frontiers

Feeding *ad libitum* without any treatment delayed biochemical, histological and functional recovery from acute hemorrhagic pancreatitis. So, it is a hot research topic to find the most favorable strategy for the treatment of acute hemorrhagic pancreatitis to improve biochemical and histological alterations, and pancreatic secretory function.

Innovations and breakthroughs

It is generally accepted that enteral nutrition may improve outcome in patients with AP if given early. However, feeding *ad libitum* without any treatment delayed biochemical, histological and functional recovery from acute hemorrhagic pancreatitis. Pancreatic rest made by blocking cholecystokinin (CCK)-1 receptor improved biochemical and histological alterations except pancreatic secretory function and HOMA-β-cell score. Pancreatic stimulation caused by eliciting endogenous CCK release from an early stage after acute pancreatitis significantly improved biochemical and histological alterations, and recovered pancreatic insulin content and HOMA-β-cell score, but slightly delayed the recovery of exocrine secretory function. The favorable treatment strategy for AP is to keep the pancreas at rest during the early stage, followed by pancreatic stimulation by promoting endogenous CCK release.

Applications

These results suggest that high-protein meals should be avoided during the early stages after AP but protein meals may be important during later times to stimulate recovery of pancreatic function.

Terminology

CCK is secreted by I cells of the upper small intestine. Its secretion is stimulated by the introduction of hydrochloric acid or fatty acids into the stomach or the duodenum. CCK stimulates the gallbladder to contract and release stored bile into the intestine. It also stimulates the secretion of pancreatic juice rich in digestive enzymes and may induce satiety. Two types of CCK receptors (type A, "alimentary," and type B, "brain") have been identified on a pharmacological basis. The CCK-A receptor was first characterized in pancreatic acini from rodents, whereas the CCK-B receptor was first found in the brain. Based on recommendations of the International Union of Pharmacology Committee regarding receptor nomenclature and drug classification, the CCK-A receptor has been renamed CCK1 receptor, and the CCK-B receptor has been renamed CCK2 receptor. CCK1 receptor binds and responds to sulfated CCK with a 500-1000-fold higher affinity or potency than sulfated gastrin or nonsulfated CCK. The CCK2 receptor binds and responds to gastrin or CCK with almost the same affinity or potency and discriminates poorly between sulfated and nonsulfated peptides. In the periphery, the CCK2 receptor is considered as the gastrin receptor.

Peer-review

This is a very good manuscript that addresses an important problem, namely how to best manage patients with acute pancreatitis. There is no specific pharmacotherapy for this disease; thus, management of patients is critically important. The authors demonstrate in a rat model of acute pancreatitis that rest followed by stimulation of CCK secretion is the most effective protocol for recovery from this disease.

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