EXPERIMENTAL ACUTE PANCREATITIS INDUCED BY EXCESSIVE DOSES OF CAERULEIN IN RATS : PROTECTIVE AND THERAPEUTIC EFFECTS OF TRYPSIN INHIBITOR URINASTATIN AND CCK RECEPTOR…

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EXPERIMENTAL ACUTE PANCREATITIS INDUCED BY EXCESSIVE DOSES OF CAERULEIN IN RATS: PROTECTIVE AND THERAPEUTIC EFFECTS OF TRYPSIN INHIBITOR URINASTATIN AND CCK RECEPTOR ANTAGONIST CR1392

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INDEXING WORDS

caerulein; experimental acute pancreatitis; trypsin inhibitor; cholecystokinin (CCK)-receptor antagonist

SYNOPSIS

First, a new experimental model of acute pancreatitis was produced in rats by subcutaneous injections of supramaximal doses of caerulein, a synthetic analogue of cholecystokinin (CCK). Four subcutaneous injections of 20 μg/kg body weight of caerulein markedly increased of serum amylase, lipase, and anionic trypsin-(ogen) levels, which peaked at 4-6 hr after the first caerulein injection. Remarkable interstitial edema of the pancreas and various numbers and sizes of cytoplasmic vacuoles in acinar cells were seen at the early time points (4-6 hr), and cellular infiltration at later periods (9-12 hr). These light microscopic findings almost completely disappeared after 24 hr. The electron microscopic examination, however, revealed that vacuoles and destructive changes in the cytoplasmic organelles in acinar cells were still detectable even after 24 hr and these structural alterations disappeared after 7 days. Secondly, we examined the therapeutic and protective effects of urinary trypsin inhibitor, uri-

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nastatin, and of the CCK receptor antagonist, CR1392, on caerulein-induced acute pancreatitis. Five intraperitoneal injections of 50,000 U/kg body weight urinastatin inhibited the increase of serum amylase activity and lessened histologic evidence of interstitial edema, vacuolization, and inflammation. Pretreatment with CR1392 abolished the elevation of serum amylase activity and completely alleviated the histologic alterations. CR1392, even when given after the induction of acute pancreatitis (post-treatment), reduced the increase of serum amylase activity and the degree of cytoplasmic vacuolization.

INTRODUCTION

It has been generally thought that acute pancreatitis begins with the extravasation of pancreatic digestive enzymes from acinar cells and their activation in the intraparencymal tissue leading to the autodigestion of the pancreas.[2, 4] In contrast, recent studies have suggested that the blockade of exocytosis and intracellular activation of digestive enzymes by lysosomal hydrolases are likely causes of some experimental models of acute pancreatitis.[14, 23, 25] Interstitial and edematous pancreatitis with a low mortality rate is the most frequent type of acute pancreatitis in Japan.[13] However, there were no ideal models with interstitial and edematous pancreatitis, since most of the experimental models previously reported were lethal acute hemorrhagic and necrotizing pancreatitis.[1, 14, 19]

Pancreatic enzyme secretion caused by cholecystokinin (CCK) or caerulein (an analogue of CCK) is characterized by a biphasic dose-response curve.[7, 10, 22] With increasing concentrations of these secretagogues, the secretion increases, becomes maximal, and then decreases with supramaximal concentrations. The present study has demonstrated that subcutaneous injections of supramaximal doses of caerulein induce acute interstitial pancreatitis in rats. In addition, the beneficial effects of the trypsin inhibitor, urinastatin, extracted from human urine, and a new CCK receptor antagonist, CR1392, were examined in this experimental model of acute pancreatitis.

MATERIALS AND METHODS

Animals

Male Wistar rats, weighing 200-280 g, were used in all experiments. The animals were kept at 22 ± 1C on a 12-light and dark cycle with free access to water and a standard rat chow (Oriental Yeast Co., Tokyo, Japan).

Experimental protocol

1. Experimental acute pancreatitis

Four subcutaneous injections of 5, 10, 20, or 50 μg/kg body weight of caerulein (Ceosunin®, Kyowa Hakko, Ltd., Tokyo, Japan) were given to rats at hourly intervals over 3 hr. Control rats received the same volume of 0.15 M NaCl solution in a similar manner. All animals were fed ad libitum until killing. Blood samples were collected from the jugular vein under light ether anesthesia 9 hr after the first caerulein injection. All rats were sacrificed by decapitation; the pancreas was rapidly removed, freed from fat and lymph nodes, and weighed. Since 20 μg/kg body weight of caerulein was found to be the minimal effective dose that induced the biochemical and histologic alterations of acute pancreatitis, experiments evaluating the time course of caerulein-induced pancreatitis and the effects of the trypsin inhibitor urinastatin and CCK-receptor antagonist CR1392 on acute pancreatitis were performed by injecting 20 μg/kg body weight of caerulein or 0.15 M NaCl subcutaneously 4 times at hourly intervals. In these experiments, rats were killed at 4, 5, 6, 9, 12, 18 and 24 hr, and 7 and 14 days after the first caerulein injection. Blood was collected before killing and the pancreas was removed at the time indicated.

2. Protective effect of the trypsin inhibitor urinastatin

Rats with acute pancreatitis were given either urinastatin (UTI; Miraclid®, Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) in a dose of 50,000 units/kg body weight dissolved in 0.15 M NaCl, or the same volume of 0.15 M NaCl solution, through five intraperitoneal injections at hourly intervals from 30 min before the first caerulein injection to 30 min after the last injection. All animals were sacrificed at 6, 9, and 24 hr after the first

caerulein injection. Blood was collected and the pancreas was removed at the time indicated.

3. Protective and therapeutic effects of the CCK receptor antagonist CR1392

CR1392 (a generous gift from Rotta Research Laboratorium S.P.A. Milano, Italy) was administered in a dose of 10 or 50 mg/kg body weight as a single subcutaneous injection either 30 min before the first caerulein injection (protective effect) or 30 min after the last injection (therapeutic effect). Control experiments were performed by injecting the same volume of 0.15 M NaCl solution. All animals were sacrificed at 6, 9, and 24 hr after the first caerulein injection. Blood was collected and the pancreas was removed at the time indicated.

Histologic examination

A portion of the pancreas was fixed overnight in 10% formaldehyde solution. Tissues were dehydrated in a graded ethanol series, embedded in paraffin, sectioned into 5 μm slices, and stained with hematoxylin and eosin. Slides were coded and examined blindly by the pathologist for grading of histologic alterations. The histologic grading of vacuolization and necrosis was based on the approximate percentage of cells involved; 0=absent, 1=less than 5%, 2=5-25%, 3=25-50%, 4=more than 50%. Grading of the interstitial edema and inflammatory changes was made using a scale ranging from 1 to 4 for minimal to maximal alterations.

For electron microscopy, tissues were fixed with cold 1% glutaraldehyde in a 0.1 M phosphate buffer. Tissues were then postfixed with 1% OsO_4 in 0.1 M phosphate buffer and wahsed with several changes of 0.1 M phosphate buffer, dehydrated in a graded series of ethanol, and embedded in Epon 812 mixture. Sections 600-700 Å thick were cut using a Leitz ultratome and doubly stained with uranyl acetate and lead nitrate, and then examined with a Hitachi EM electron microscope.

Assay

A portion of pancreatic tissue (about 200 mg wet weight) was homogenized in 0.15 M NaCl using a motor-driven, Teflon-coated glass homogenizer. Protein was determined by the method of Lowry

et al[15] with bovine plasma albumin as a standard. DNA was measured fluorometrically by the reaction between 3, 5-diaminobenzoic acid and deoxyribose sugar using calf thymus DNA (type I, Sigma Chemical Co., St, Louis, MO) as a standard.[12] Amylase activity in serum and pancreatic homogenates was determined by a chromogenic method with the Phadebas amylase test[3] and expressed in Somogyi units (SU). Tryspinogen levels in pancreatic homogenates were determined following activation with enterokinase (Sigma Chemical Co., St. Louis MO) and measured by the method of Erlanger et al[6] and expressed in BAEE units (U). Lipase activity in serum and pancreatic homogenates was determined according to Whitakar using α-naphtyl palmitate as a substrate and expressed in International units.[26] Serum anionic trypsin(ogen) was determined by radioimmunoassay according to the method described by Tsukamoto et al.[24] Purified rat anionic trypsin and rabbit antisera were generous gifts from Dr. Tsukamoto of the Biochemical Research Laboratory, Martinez VA Medical Center. Rabbit antisera to rat anionic trypsinogen was used at a final dilution of 1:320,000. An immobilized form of goat anti-rabbit IgG (Immunobeads, Bio-Rad Laboratories, Richmond, CA) was used to separate bound and free labeled antigen following 24 hr incubation at 4C.

Data analysis

Comparison of the difference between mean values of the various groups of experiments was made by analysis of variance or the Wilcoxon rank sum test. A difference with a P value of less than 0.05 was considered statistically significant. Results are expressed as the mean ± standard error (SE).

RESULTS

Effects of different doses of caerulein

Subcutaneous injections of 10, 20, and 50 μg/kg body weight of caerulein produced marked increases in serum amylase activity with 2-, 7-, and 8-fold elevation, respectively, over the values in control rats at 9 hr after the first caerulein injection (Table 1). The injections of the two highest doses of caerulein induced remarkable increases in pancreatic wet weight as well as serum amylase activity, and exerted the histologic evidence of acute

interstitial pancreatitis. A dose of 20 μg/kg body weight of caerulein, however, caused near-maximal damage that was not significantly different from that induced by 50 μg/kg body weight of caerulein. Marked interstitial edema, cytoplasmic vacuoles in acinar cells, and cellular infiltration in the interstitial space were seen in the pancreata of rats injected with 20 and 50 μg/kg body weight of caerulein. Based on these observations, 20 μg/kg body weight of caerulein was chosen to evaluate the time course of pancreatitis and the beneficial effects of urinastatin and CR1392 on caerulein-induced acute pancreatitis.

Table 1 Serum amylase activity, pancreatic wet weight, and histologic alterations of the pancreas after 4 subcutaneous injections of different doses of caerulein.

Dose of caerulein (μg/kg body weight)	Serum amylase activity (SU/100ml)	Pancreatic wet weight (mg)	Histologic alterations			
			Interstitial edema	Vacuolization	Inflammation	Necrosis
0 (8)	5737 ± 231	1028 ± 32	0	0	0	0
5 (4)	8067 ± 1214	862 ± 43	0-1 (0.5 ± 0.3)	1 (1)	0-1 (0.3 ± 0.3)	0-1 (0.3 ± 0.3)
10 (4)	11379 ± 2245*	975 ± 76	1-2 (1.5 ± 0.3)	1-2 (1.5 ± 0.3)	1-2 (1.5 ± 0.3)	0-1 (0.8 ± 0.3)
20 (6)	39690 ± 3969*	1320 ± 100*	2-3 (2.8 ± 0.2)	2-4 (3.2 ± 0.3)	2-4 (3.0 ± 0.5)	1 (1)
50 (4)	43068 ± 4411*	1606 ± 233*	2-4 (3.0 ± 0.4)	2-4 (3.3 ± 0.2)	2-4 (3.0 ± 0.4)	1 (1)

All rats received a total of 4 subcutaneous injections of saline or caerulein at hourly intervals over 3 hr and they were killed 9 hr after the first injection. The histologic grading is carried out as indicated in Materials and Methods and is shown by ranges. Each value represents the mean ± SE of the number of rats indicated in parentheses. *Significant difference ($p<0.05$) vs. control rats with saline injections (0 μg/kg body weight).

Time course of caerulein-induced acute pancreatitis

Four subcutaneous injections of 20 μg/kg body weight of caerulein induced maximal increases in serum amylase and lipase activities of 20- and 400-fold, respectively, over the basal value at 6 hr after the first caerulein injection (Fig. 1). Thereafter the activity of these enzymes decreased to normal values after 24

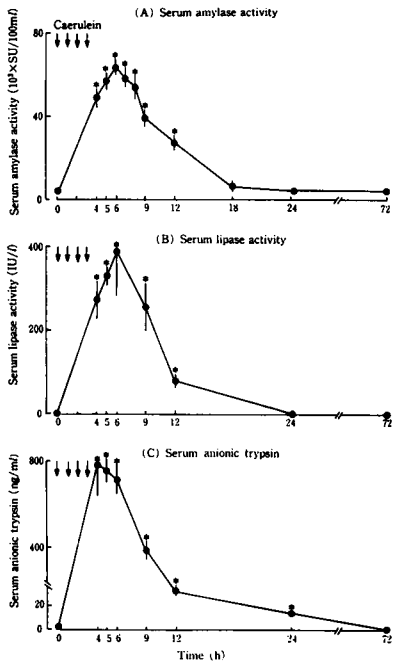

Fig. 1
Time course of serum amylase, lipase and anionic trypsin(ogen) levels in rats treated with 4 subcutaneous injections of 20 μg/kg body weight of caerulein. Data represent means ± SE of 5-10 animals. *Significant difference ($p<0.05$) vs. control (0 hr).

hr. On the other hand, anionic trypsin(ogen) levels had already peaked at 4 hr and remained elevated even 24 hr after, but returned to normal after 3 days.

The pancreata of caerulein-treated rats appeared grossly swollen and enlarged. Pancreatic wet weight increased as much as 100% at 4 hr after the first injection, and then decreased gradually (Table 2). Caerulein induced no significant changes in the pancreatic DNA content at any time point, whereas protein content significantly decreased at 24 hr after the first caerulein injection.

Four subcutaneous injections of caerulein produced the histologic evidence of acute pancreatitis in rats. Interstitial edema with dilated vessels was a prominent histologic feature at early time points (Fig. 2B, 3A). Cytoplasmic vacuoles of various numbers and sizes could be seen in the pancreatic acinar cells (Fig. 2A, 3B), whereas inflammatory changes in the interstitial space were less frequent findings. The reduction in numbers of intracellular zymogen granules, swollen mitochondria, and vacuoles in the cytoplasm were frequent findings under the electron microscope (Fig. 4A). These cytoplasmic vacuoles, which fused with each other or with zymogen granules, contained amorphous materials with

Table 2 Time course of serum amylase activity, pancreatic wet weight, and protein and DNA content in rats treated with 20 μg/kg body weight of caerulein.

Time after the start of caerulein injection	Pancreatic wet weight (mg)	Content in the pancreas		
		Protein (mg/pancreas)	DNA (mg/pancreas)	Protein/DNA (mg/mg)
0 h (8)	1028 ± 32	152.6 ± 4.3	4.8 ± 0.6	34.7 ± 4.1
4 h (5)	2056 ± 229*	143.1 ± 6.9	4.5 ± 0.6	34.2 ± 4.1
5 h (5)	1501 ± 173*	148.7 ± 12.4	4.9 ± 0.3	30.7 ± 2.2
6 h (10)	1492 ± 97*	153.1 ± 7.4	4.9 ± 0.3	33.9 ± 2.0
9 h (10)	1338 ± 85*	147.8 ± 12.7	4.7 ± 0.3	31.0 ± 1.4
12 h (6)	1316 ± 90*	135.2 ± 10.5	5.4 ± 0.4	29.9 ± 1.4
18 h (6)	1109 ± 47	146.6 ± 12.8	5.3 ± 0.3	26.7 ± 3.8
24 h (6)	933 ± 65	113.8 ± 11.2*	5.3 ± 0.3	20.8 ± 2.1*
7 d (8)	915 ± 21*	142.0 ± 5.0	4.1 ± 0.1	34.8 ± 1.3
14 d (5)	1184 ± 26	181.0 ± 8.8	5.2 ± 0.3	35.7 ± 1.2

All values are means ± SE of the number of rats indicated in parentheses. *Significant difference vs. control (0 hr) ($p<0.05$).

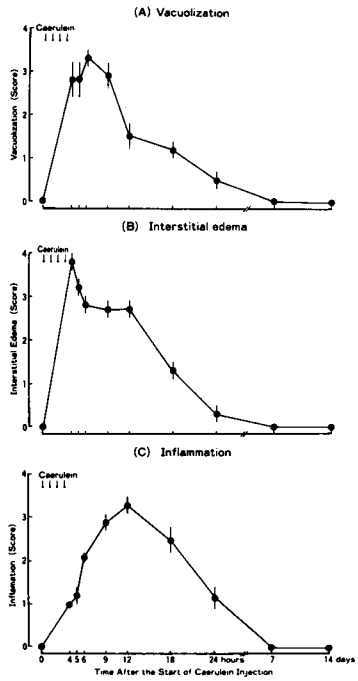

Fig. 2
Time course of histologic alterations in rats treated with 4 subcutaneous injections of 20 μg/kg body weight of caerulein. Data represent means ± SE of the histologic grading carried out as indicated in Materials and Methods of 5-10 animals.

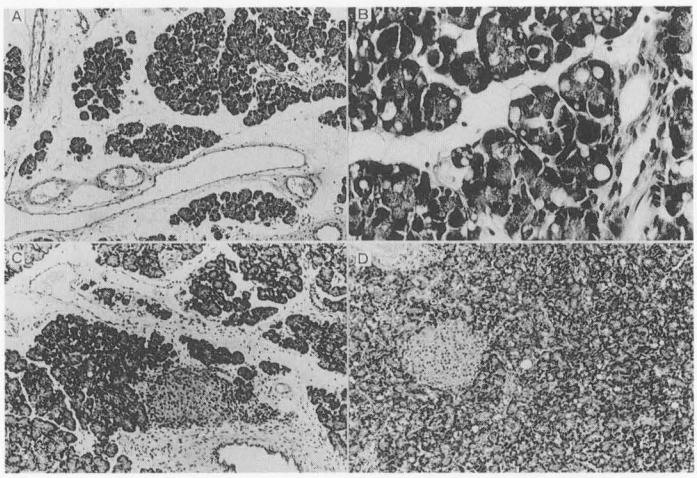

Fig. 3 Light micrographs of the pancreas in rats treated with 4 subcutaneous injections of 20 μg/kg body weight of caerulein. (A) Pancreas 4 hr after the first caerulein injection. Remarkable interstitial edema with dilated vessels can be seen. (x 150). (B) Pancreas 6 hr after the first caerulein injection. Various sizes and numbers of cytoplasmic vacuoles are present in the acinar cells. (x 280). (C) Pancreas 12 hr after the first caerulein injection. Inflammatory cells consisting mainly of neutrophils markedly infiltrate the interstitial space. (x 77). (D) Pancreas 24 hr after the first caerulein injection. Almost normal architecture with few cytoplasmic vacuoles and cellular infiltration is observed. (x 77).

various electron densities (Fig. 4B, 4C). Basolateral exocytosis of vacuoles discharging their contents into the interstitial space was observed (Fig. 4D), whereas normotopic exocytosis at the luminal plasma membrane was markedly reduced. Interstitial edema and cytoplasmic vacuolization of acinar cells gradually subsided, whereas cellular infiltration of inflammatory cells was seen not only in the perivascular space of the interstitium but also between acinar cells after 12 hr (Fig. 2C, 3C). Cellular degeneration and necrosis were rarely observed at any time point.

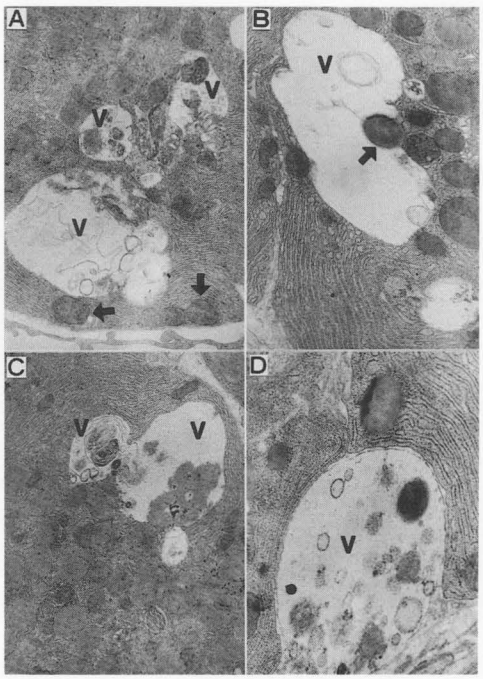

Fig. 4 Electron micrographs of pancreatic acinar cells in rats treated with 4 subcutaneous injections of 20 μg/kg body weight of caerulein. (A) Pancreatic acinar cell 4 hr after the first caerulein injection. Large cytoplasmic vacuoles (V), which contain amorphous material of various densities, and swollen mitochondria (arrow) are seen. Zymogen granules are scarce. (x 11,800). (B) Pancreatic acinar cell 6 hr after the first caerulein injection. A vacuole fuses with a zymogen granule and takes it up (arrow). (x 16,000). (C) Pancreatic acinar cells 6 hr after the first caerulein injection. Vacuoles fuse with each other. (x 7,600). (D) Pancreatic acinar cells 6 hr after the first caerulein injection. The exocytosis of a vacuole without the complete succession of the limiting membrane is observed at the basolateral plasma membrane (x 12,800).

Under the light microscope these histologic alterations had almost completely disappeared 24 hr after the first caerulein injection (Fig. 3D). However, the degradation and decrease in number of zymogen granules in the acinar cells were still detectable by electron microscopic examination after 24 hr. These histologic alterations completely disappeared within 7 days.

Effect of urinastatin (UTI)

Although UTI treatment could not inhibit the increase of serum amylase activity at early time points (4-7 hr), serum amylase levels at 8 and 9 hr after the first caerulein injection were significantly reduced by UTI treatment (Fig. 5). Pancreatic wet weight in rats treated with UTI was significantly increased at 6 hr after the first caerulein injection, to a level similar to that in the control rats. The increased pancreatic weight returned to normal at 9 hr after UTI treatment (Table 3). Pancreatic protein cotent in UTI-treated rats did not differ from that in control rats. The histologic signs of acute pancreatitis were also greatly alleviated by intraperitoneal injections of UTI. The vacuolization process in acinar cells at 6 and 9 hr after the first caerulein injection was significantly suppressed by UTI. UTI treatment could not inhibit interstitial edema and inflammatory changes at 6 hr as can be supported by the unaltered pancreatic wet weight. UTI, however, significantly reduced the degrees of these histologic alterations at later time points.

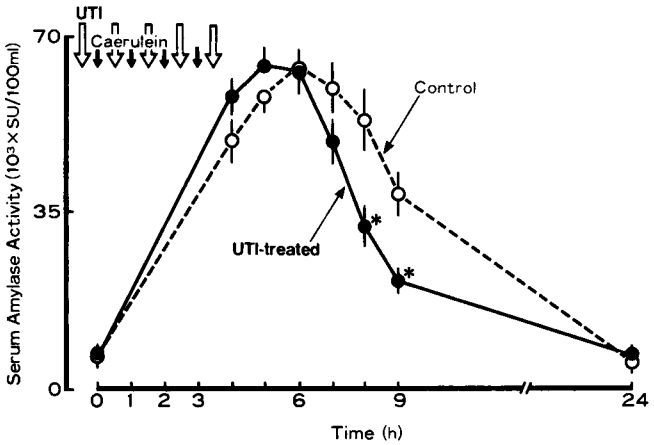

Fig. 5 Effect of trypsin inhibitor, urinastatin (UTI), on serum amylase activity in caerulein-induced acute pancreatitis. All rats received 4 subcutaneous injections of 20 μg/kg body weight of caerulein with 5 intraperitoneal injections of saline (control) or UTI (UTI-treated). Data represent means ± SE of 5-8 animals. *Significant difference ($p<0.05$) vs. control rats at the corresponding time.

Table 3 Effect of urinastatin (UTI) on pancreatic wet weight, protein content, and histologic alterations in caerulein-induced acute pancreatitis.

Time after the start of caerulein injection	Pancreatic wet weight(mg)	Pancreatic protein content(mg/pancreas)	Histologic alterations		
			Edema	Vacuolization	Inflammation
0 h (7)	1041 ± 34	152.6 ± 4.3	0	0	0
6 h					
Control (8)	1568 ± 104†	169.0 ± 8.5	2-3 (2.7 ± 0.2)	3-4 (3.1 ± 0.1)	2-3 (2.1 ± 0.1)
UTI-treated (5)	1571 ± 86†	158.7 ± 7.4	2-3 (2.4 ± 0.2)	1-2 (1.6 ± 0.2)*	1-2 (1.2 ± 0.2)
9 h					
Control (7)	1367 ± 116†	148.7 ± 16.9	2-3 (2.7 ± 0.2)	1-4 (2.6 ± 0.4)	2-4 (3.0 ± 0.2)
UTI-treated (5)	1039 ± 36	145.6 ± 11.2	1-2 (1.4 ± 0.2)*	1 (1)*	0-1 (0.8 ± 0.2)*
24 h					
Control (6)	955 ± 21	113.8 ± 11.2	0-1 (0.3 ± 0.2)	0-1 (0.6 ± 0.3)	1-2 (1.6 ± 0.3)
UTI-treated (5)	791 ± 41*†	110.8 ± 4.5†	0 (0)	0-1 (0.4 ± 0.2)	0-1 (0.6 ± 0.2)

All rats received 4 subcutaneous injections of 20 μg/kg body weight of caerulein with 5 intraperitoneal injections of saline (control) or UTI (UTI-treated) from 30 min before the first caerulein injection to 30 min after the final caerulein injection. Data represent means ± SE of the number of rats indicated in parentheses. *Significant diffrence ($p<0.05$) vs. control at corresponding time. †Significant difference ($p<0.05$) vs. rats without any treatments (0 hr).

Effect of CR1392

Pretreatment with CR1392 dose-dependently inhibited the increase in serum amylase activity induced by excessive doses of caerulein (Fig. 6A). CR1392 at a dose of 50 mg/kg body weight completely abolished the increase in pancreatic wet weight as well as serum amylase activity. A single subcutaneous injection of CR1392 caused a significant reduction in pancreatic protein content at 6 and 9 hr compared with control rats not receiving CR1392 (Table 4). Histologic examination showed that pretreatment with 50 mg/kg body weight of CR1392 completely suppressed the induction of interstitial edema after caerulein injections. The

Table 4 Effect of pretreatment with CR1392 on pancreatic wet weight, protein content, and histologic alterations in caerulein-induced acute pancreatitis.

Time after the start of caerulein injection	Pancreatic wet weight(mg)	Pancreatic protein content(mg/pancreas)	Histologic alterations		
			Edema	Vacuolization	Inflammation
0 h (4)	980 ± 42	148.9 ± 9.7	0	0	0
6 h					
Control (6)	1595 ± 118†	169.5 ± 10.4	3 (3)	3-4 (3.3 ± 0.2)	2 (2)
CR1392 pretreated (4)	840 ± 59*	91.8 ± 9.1*†	0 (0)*	2-4 (3.0 ± 0.3)	1-2 (1.3 ± 0.3)
9 h					
Control (6)	1378 ± 96†	154.2 ± 17.6	2-3 (2.7 ± 0.2)	1-3 (2.2 ± 0.3)	2-4 (3.0 ± 0.3)
CR1392 pretreated (4)	845 ± 44*	110.3 ± 11.7*†	0 (0)*	0-1 (0.5 ± 0.2)*	0-1 (0.3 ± 0.3)*
24 h					
Control (4)	895 ± 59	116.7 ± 15.7	0-1 (0.4 ± 0.3)	0-1 (0.6 ± 0.3)	1-3 (1.8 ± 0.4)
CR1392 pretreated (4)	858 ± 41	110.3 ± 11.7†	0 (0)	0 (0)	0 (0)

All rats received 4 subcutaneous injections of 20 μg/kg body weight of caerulein pretreated with saline (control) or 50 mg/kg body weight of CR1392 (CR1392 pretreated) 30 min before the first caerulein injection. Data represent means ± SE of the number of rats indicated in parentheses. *Significant difference ($p<0.05$) vs. control at corresponding time. †Significant difference ($p<0.05$) vs. rats without any treatments (0 hr).

degree of vacuolization and cellular infiltration at 9 hr were also greatly alleviated by the pretreatment with a single subcutaneous injection of CR1392.

A single subctaneous injection of 50 mg/kg body weight of CR1392 30 min after the last caerulein injection also appeared to inhibit the increase of serum amylase activity (Fig. 6B). CR1392 when given after the induction of acute pancreatitis could not reduce the increase in pancreatic wet weight at early time points (Table 5), but significantly reduced it at 9 hr after the caerulein injections. CR1392, irrespective of whether given before the start of caerulein injection or after the last injection, reduced pancreatic protein content. Histologic alterations at early time points such as interstitial edema and dilated vessels

were only slightly affected by CR1392. However, the degree of cytoplasmic vacuolization in the acinar cells at 9 hr was significantly suppressed. Interstitial edema and the infiltration of inflammatory cells were also reduced later by post-treatment with CR1392.

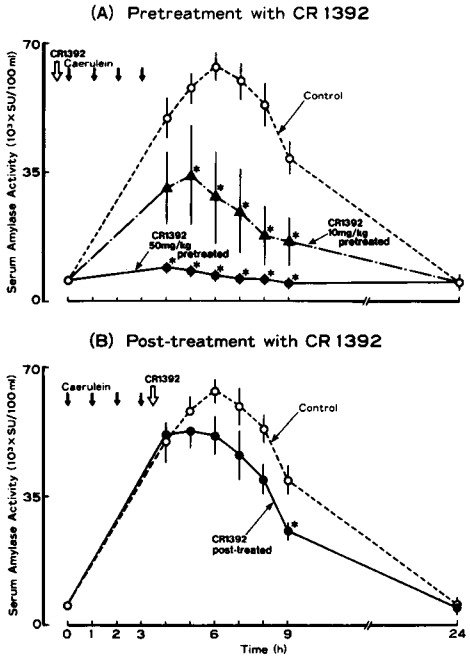

Fig. 6
Effect of pre- (A) and post-treatment (B) with CR1392 on serum amylase activity in caerulein-induced acute pancreatitis. All rats received 4 subcutaneous injections of 20 μg/kg body weight of caerulein and pre- or post-treated with a single subcutaneous injections of saline (control) or CR1392. Data represent means ± SE of 4-6 animals. *Significant difference ($p<0.05$) vs. control rats at the corresponding time.

DISCUSSION

The present study demonstrated that four subcutaneous injections of supramaximal doses of caerulein, an analogue of CCK, increased both serum pancreatic digestive enzyme levels and pancreatic wet weight, and produced histologic evidence of mild acute interstitial pancreatitis in rats. This experimental pancreatitis is characterized by the presence of various sizes and numbers of cytoplasmic vacuoles in acinar cells and interstitial edema at early time points, and cellular infiltration consisiting mainly of neutrophils at later periods. In addition, the degeneration and necrosis of exocrine cells were only rarely seen at any time points. These histologic alterations resemble to a great extent clinical acute pancreatitis in Japan where acute interstitial and edematous pancreatitis is more frequent than lethal necrotizing

Table 5 Effect of post-treatment with CR1392 on pancreatic wet weight, protein content, and histologic alterations in caerulein-induced acute pancreatitis.

Time after the start of caerulein injection	Pancreatic wet weight(mg)	Pancreatic protein content(mg/pancreas)	Histologic alterations		
			Edema	Vacuolization	Inflammation
0 h (6)	1075 ± 42	167.7 ± 9.7	0	0	0
6 h					
Control (6)	1540 ± 87†	165.2 ± 8.1	2-3 (2.8 ± 0.2)	3-4 (3.2 ± 0.2)	2-3 (2.2 ± 0.2)
CR1392 post-treated (4)	1378 ± 234†	127.8 ± 18.9	1-3 (2.3 ± 1.5)	1-3 (2.3 ± 0.5)	2-4 (3.0 ± 0.4)
9 h					
Control (6)	1409 ± 85†	170.7 ± 13.7	2-3 (2.7 ± 0.2)	2-4 (2.8 ± 0.3)	2-4 (3.0 ± 0.3)
CR1392 post-treated (4)	1051 ± 88*†	109.9 ± 11.0*†	1-3 (2.3 ± 0.5)	1-2 (1.5 ± 0.3)*	1-3 (1.8 ± 0.5)
24 h					
Control (5)	881 ± 48†	111.1 ± 13.4†	0-1 (0.5 ± 0.3)	0-1 (0.8 ± 0.3)	1-2 (1.5 ± 0.3)
CR1392 post-treated (4)	758 ± 25†	108.7 ± 16.3†	0 (0)	1-2 (1.5 ± 0.3)	0-1 (0.3 ± 0.3)

All rats received 4 subcutaneous injections of 20 μg/kg body weight of caerulein with saline (control) or 50 mg/kg body weight of CR1392 (CR1392 post-treated) 30 min after the final caerulein injection. Data represent means ± SE of the number of rats indicated in parentheses. *Significant difference ($p<0.05$) vs. control at corresponding time. †Significant difference ($p<0.05$) vs. rats without any treatments (0 hr).

and hemorrhagic pancreatitis.[13]

It has been suggested that the vacuoles in the acinar cells were formed as a result of the impaired separation of zymogen granules and lysosomal hydrolases,[23] which was also noted during the induction of acute pancreatitis induced by feeding young female mice a choline-deficient diet supplemented with ethionine.[14] Immunohistochemical studies revealed that these vacuoles contained both digestive enzymes and lysosomal hydrolases.[25] These observations suggest taht the intracellular activation of trypsinogen to trypsin by lysosomal enzymes might occur, leading to the autodigestion of acinar cells. Moreover, the basolateral exocytosis of cytoplasmic vacuoles was observed under the electron microscope, whereas the normal discharge of zymogen granules at the luminal plasma membrane was reduced. These phenomena indicate

that the contents of the vacuoles were discharged into the interstitium, inducing interstitial edema of the pancreas. However, since the degree of cytoplasmic vacuolization did not always correlate with that of interstitial edema in the present study, the phenomenon of the basolateral exocytosis may not be the principal cause of edema. The presence of the dilated vessels and marked perivascular infiltration strongly suggest the increased vascular permeability,[11] which may account for the interstitial edema of the pancreas.

The time course of serum amylase, lipase, and anionic trypsin(ogen) levels after caerulein injections almost correlated with the histologic alterations of interstitial edema and cytoplasmic vacuolization in the pancreas. There was, however, a distinct dissociation between the rise of digestive enzymes levels in the serum and cellular infiltration into the interstitial space. After 24 hr serum amylase and lipase activities returned to normal values but anionic trypsin(ogen) levels still remained 3 times higher than the preloading level. At this time point light microscopic studies showed almost normal architecture, whereas electron microscopic examination revealed the destructive changes in intracellular organelles. Therefore, serum anionic trypsin(ogen) levels better represent the structural alterations of acinar cells than do other digestive enzymes.

The present study has demonstrated the first evidence that the urinary trypsin inhibitor urinastatin (UTI) protects against the biochemical and histologic symptoms of caerulein-induced acute pancreatitis in rats. UTI is an acid glycoprotein extracted from human urine with a molecular weight of 67,000 and contains 5-12% of neutral sugar.[18] Although UTI could not inhibit the rise of serum amylase activity during the first 4-7 hr, it did so later as well as alleviating the histological evidence of acute pancreatitis. Taken together, UTI does not block the induction of acute pancreatitis induced by caerulein but does reduce its severity, leading to rapid recovery from the disease.

Since the activation of trypsinogen in the pancreas is reported to be the trigger of acute pancreatitis, various kinds of trypsin inhibitors have been developed in the hope of treating acute pancreatitis. Contrary to UTI, aprotinin has conferred no therapeutic advantages against acute pancreatitis in man or in

animal models.[5, 17] UTI has a broader spectrum than does aprotinin by its additional capacity to inhibit α-chymotrypsin, elastase, lipase, carboxypeptidase, and so on.[8, 9] This may account for their different protective effects against acute pancreatitis, because trypsin is not the only enzyme involved in the pathogenesis and development of this disease.[2, 4]

It has been reported that CCK appears to contribute to the development of pancreatitis, and the CCK receptor antagonist proglumide improves the survival rate and ameliorates the histologic alterations in acute hemorrhagic pancreatitis induced by feeding mice a choline-deficient ethionine-supplemented diet.[20] The proglumide derivative CR1392 is several hundred times more potent than proglumide.[16, 21] The present study indicates that the pretreatment with 50 mg/kg body weight of CR1392 almost completely blocks the induction of acute pancreatitis by caerulein. Moreover, CR1392 even when given after the induction of pancreatitis significantly inhibited the increase of serum amylase activity and the degree of cytoplasmic vacuoles at 9 hr after the first caerulein injection. This suggests that blocking the CCK receptor, and thereby inhibiting the stimulation of enzyme secretion, may be also beneficial in human acute pancreatitis. These CCK receptor antagonists may become new therapeutic agents against acute pancreatitis.

The present study shows the course of biochemical and structural alterations in the acute pancreatitis induced by supramaximal caerulein stimulation. This experimental model of mild acute interstitial pancreatitis is easy to make, highly reproducible, and noninvasive. It might contribute to studies not only of the pathophysiological mechanism of acute pancreatitis but also of the therapeutic value of new agents.

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