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Analysis of zinc and other elements in rat pancreas, with studies in acute pancreatitis

Mikiya Kashiwagi,[1] Hiroshi Akimoto,[1] Junko Goto,[2] and Teruaki Aoki[1]

[1] Department of Surgery, Jikei University, School of Medicine, 3-25-8, Nishishinbashi, Minato-ku, Tokyo, 105 Japan
[2] Department of Textile and Clothing, Kyoritsu Women's University, 2-2-1, Hitotsubashi, Chiyoda-ku, Tokyo, 101 Japan

Abstract: Determination of the concentration of certain elements makes it possible to investigate the physiology of the pancreas. We used X-ray fluorescence to determine the concentrations of zinc and other elements in the pancreas of normal (control) rats and those with cerulein-induced pancreatitis. Ten elements (Zn, Ni, Fe, P, Ca, Cl, S, K, Ti, and Mn) were detected in controls. In the early stage of acute pancreatitis, the pancreatic concentrations of Zn, Ni, Fe, and P were significantly decreased ($P < 0.05$) and those of Ca and Cl were significantly increased ($P < 0.05$), compared with control levels. However, levels of S, K, and Ti did not differ significantly from the control values. Mn was detected in only some samples. The serum levels of Zn and Fe were significantly elevated ($P < 0.05$) in acute pancreatitis. These observations indicate that Zn and these other nine elements could play an important role in acute pancreatitis.

Key words: acute pancreatitis, X-ray fluorescence, zinc, elements

Introduction

The physiological effects of pancreatic constituents, including essential metals, have been studied, and the important role of zinc (Zn) in the pancreas has attracted much notice.[1–4] Zn is required for the formation and functioning of metalloenzymes,[1,2] and is essential for the processing, storage, and secretion of insulin.[5] In the exocrine pancreas, Zn is concentrated in the acinar cells and is secreted into the duodenum with pancreatic juice.[6,7] In experiments carried out on rats, Zn deficiency caused some degree of acinar cell degeneration,[8] while a diet with a high concentration of Zn significantly increased the survival rate of mice with acute pancreatitis.[9] These observations suggest that the pancreas may exert some control over the metabolism of Zn.

Determination of the concentrations of Zn and other elements in pancreatic tissue would be of great interest in regard to their role in pancreatic disease. To our knowledge, no investigation of metallic elements in acute pancreatitis has been performed. The aim of this study was to determine the concentrations of Zn and certain other metallic and non-metallic elements in the pancreas of rats with acute pancreatitis induced by cerulein.

While the most widely used analytical method is atomic absorption spectroscopy, this method is mainly used to study liquids such as serum or pancreatic juice.[10] Chemical analysis using ditizone as a reagent also involves certain difficulties due to the interference of other ions.[11] We therefore employed fluorescence X-ray analysis (FXA). This method makes it possible to obtain simultaneous measurements of multiple metallic and non-metallic elements and the changes in concentration of these elements in acute pancreatitis.

Materials and methods

Animals

Male Wistar rats weighing 180–230 g (Ishikawa Animal Laboratories, Saitama, Japan) were used. Rats were fed standard laboratory chow (EC-2; Clea Japan Inc., Tokyo, Japan) containing 6.48 mg Zn/100 g (by analysis) and they received tap water ad libitum for 3 weeks until the time of the experiment. The rats were housed individually in plastic cages and were kept under a constant temperature.

Offprint requests to: M. Kashiwagi
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Acute pancreatitis was induced by the intraperitoneal injection of cerulein (Kyowa Hakko Co., Tokyo, Japan) in 0.9% NaCl, given at a dose of 50 μg/kg (group A, $n = 6$) or 100 μg/kg (group B, $n = 6$) administered two times at 1-h interval. Control rats ($n = 12$) received the same volume of 0.9% NaCl by intraperitoneal injection. All animals were fasted for 16 h before the experiment, but were permitted free access to water. Two h after the last dose of cerulein, all animals were anesthetized by the intraperitoneal injection of pentobarbital sodium (50 mg/kg). Blood samples were taken and the pancreas was rapidly removed and weighed.

Preparation of samples

Each pancreas was placed in a beaker and dried at 160°C for 3 h in an oven under reduced pressure. Samples were kept in a desiccator until analysis. Each dried pancreas was weighed, carefully wrapped with the thin polyethylene film used for solid sample testing in FXA, and analyzed. We had previously determined that these samples could be kept in a desiccator for 1 week without developing an odor. In addition, preliminary data (not shown) demonstrated that reproducible FXA data could be obtained from dried organs stored in the desiccator for 1 week.

FXA measurement and blood analysis

The apparatus used in these experiments was an energy dispersive fluorescence X-ray spectrometer, Link XR-300 (Rigaku Electric Co., Tokyo, Japan), equipped with a semiconductor and multichannel analyzer. All measurements were carried out at a voltage of 20 KV and a current of 200 A for 200 s, without a filter or collimeter. The lower limit of detection varied for each element. The concentration was expressed in terms of the intensity of the emitted X-ray characteristic of each element. This could be easily measured with a scintillation counter. The concentration of each element was expressed as counts/g dry weight of the specimen. The serum amylase concentration was determined by an enzyme assay. An atomic absorption method was used to measure the serum levels of Zn and nickel (Ni).

Statistical analysis

Data are expressed as means ± SD. Comparisons between the controls and drug-treated groups were made using the two-tailed Student's *t*-test for unpaired values, with a level of $P < 0.05$ being regarded as statistically significant.

Results

All rats administered cerulein developed an edematous form of acute pancreatitis which was apparent on macroscopic examination. The dry weight of the pancreas in the groups with acute pancreatitis (groups A and B) was significantly heavier than that of the control group (0.40 ± 0.04, 0.374 ± 0.06, and 0.26 ± 0.08 mg, $P < 0.01$, respectively).

Figure 1 illustrates the typical FXA spectrum of the dried pancreas of a rat in group A. Ten elements were present: phosphorus (P), sulfur (S), potassium (K), calcium (Ca), chlorine (Cl), titanium (Ti), iron (Fe), Ni, Zn, and manganese (Mn). Other elements may also have been present at levels below the level of detection of the system. Mn was observed in 5 of 12 animals in the control group and in 4 of 6 animals in group A. The concentration of each metal was measured in terms of X-ray intensity, as shown in Fig. 1. However, the concentration was more conveniently expressed as the number of counts within the matrix. Thus, the average concentration of each element in the control group ($n = 12$) was compared with that in the acute pancreatitis groups ($n = 6$, each group), as shown in Figs 2–4. These results clearly showed that nine of these ten elements detected could be classified into three categories. The first category (Fig. 2) consisted of those influenced substantially by acute pancreatitis and decreased in concentration; Zn, Ni, Fe, and P belonged to this category. The concentration of Zn, Ni, Fe, and P in groups A and B was significantly decreased compared with the control group ($P < 0.05$). The second category (Fig. 3) included those elements not influenced by acute pancreatitis; their concentration remained virtually constant under the conditions tested; S, K,

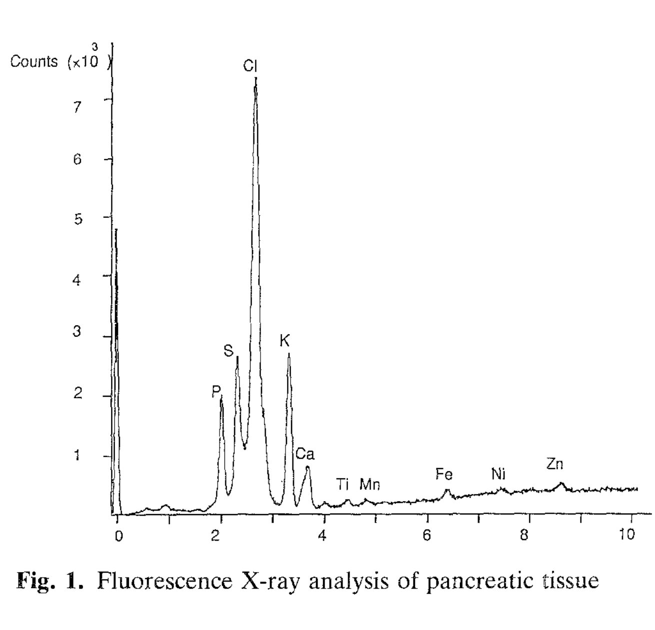

Fig. 1. Fluorescence X-ray analysis of pancreatic tissue

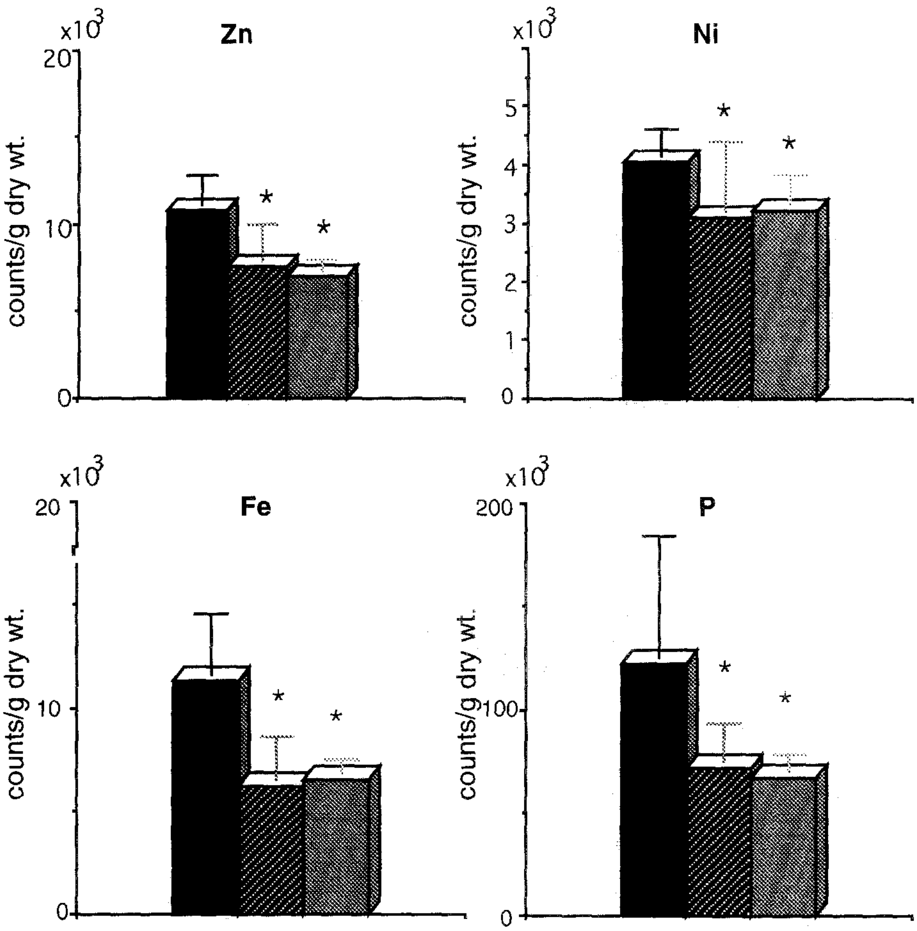

Fig. 2. Concentrations of Zn, Ni, Fe, and P in pancreatic tissue. Values are means ± SD. Differences between two groups were evaluated by unpaired Student's *t*-test. $^*P < 0.05$ compared with control. *Solid columns*, control; *striped columns*, group A (cerulein 50 μg/kg); *stippled columns*, group B (cerulein 100 μg/kg)

Table 1. Blood chemistry of rats with acute pancreatitis (groups A and B) compared with results in control group

	Amylase (IU/l)	Zn (μg/dl)	Ni (μg/dl)	Fe (μg/dl)	Ca (mg/dl)	Cl (mEq/l)	K (mEq/l)	P (mg/dl)
Control	332 ± 110	101 ± 14	0.43 ± 0.22	73 ± 16.5	9.5 ± 0.2	103 ± 2.0	4.4 ± 0.2	9.0 ± 6.0
Group A	4816 ± 918*	125 ± 16**	<0.1	111 ± 37.2*	9.5 ± 0.3	100 ± 3.5	4.6 ± 0.4	11.4 ± 1.7**
Group B	4438 ± 243*	95 ± 17	<0.1	163 ± 39.8**	9.7 ± 0.3	98 ± 3.4	4.6 ± 0.4	9.8 ± 1.4

$^*P < 0.05$; $^{**}P < 0.01$ vs control group
Values are expressed as means ± SD

and Ti belonged to this category. The third category (Fig. 4) consisted of Ca and Cl, and the concentration of Ca and Cl in groups A and B was significantly increased compared with that in the control group ($P < 0.05$).

Table 1 shows the results of serum analysis. Serum amylase and Fe levels in groups A and B were significantly elevated compared with levels in the control group ($P < 0.05$). In group A, serum levels of Zn and P were significantly higher than those in the control group ($P < 0.01$). However, serum levels of Ca, Cl, and K did not differ significantly from those in the controls.

Discussion

The biochemical and structural state of cerulein-induced acute pancreatitis in rats is consistent, reproducible, and dose-dependent. Morphological studies have shown that the pancreatic changes develop within 2 h after cerulein administration.[12] In order to investigate the early stage of acute pancreatitis in more detail, we selected the dose and injection intervals of cerulein used in this study.

FXA is employed mainly for solid specimens and enables simultaneous measurements of multiple components. FXA measurements detected ten elements, as

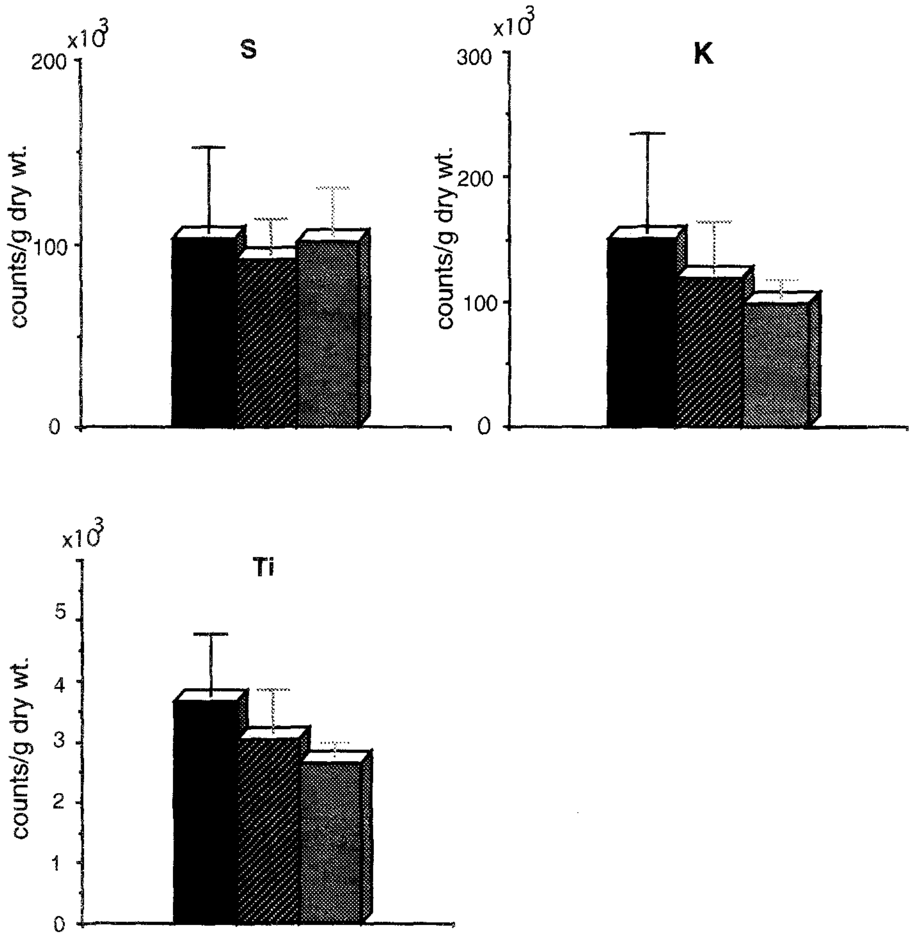

Fig. 3. Concentrations of S, K, and Ti in pancreatic tissue. Values are means ± SD. *Columns*, as in Fig. 2

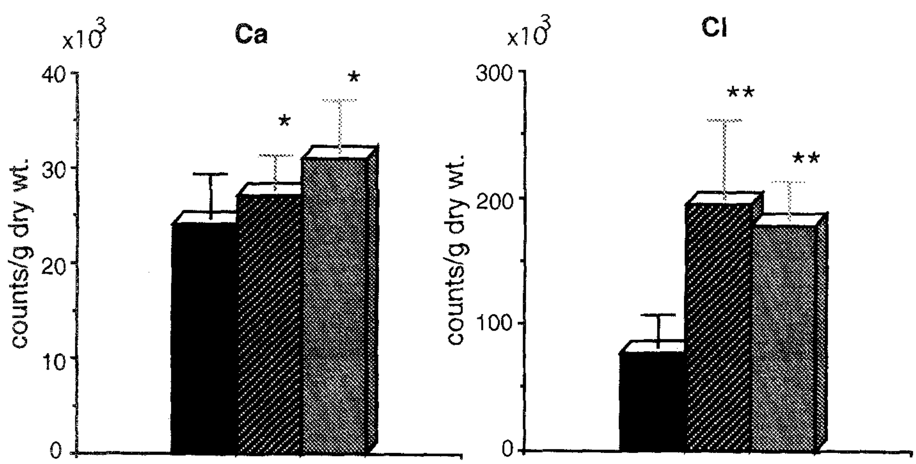

Fig. 4. Concentrations of Ca and Cl in pancreatic tissue. Values are means ± SD. Differences between two groups were evaluated by unpaired Student's *t*-test. $*P < 0.05$; $**P < 0.01$ compared with control. *Columns*, as in Fig. 2

shown in Fig. 1: P, S, K, Cl, Ca, Zn, Fe, Ni, Ti, and Mn. Mn was detected only occasionally. We consider that this was because total amounts of Mn were not sufficient to be measured by a scintillation counter, and therefore, Mn was not detected in every specimen samples. However, in humans, Mn accumulates in the pancreas more than in other organs.[13] Pancreatic juice reportedly contains at least five more metals: cadmium, lead, copper, cobalt, and chromium.[14] However, both the pancreatic juice and tissue may be shown to contain other essential elements with better analytical methods. FXA is not very sensitive to trace amounts of light elements; therefore a trace amount of a substance such as Na could have been present, but not detected.

Zn is located in the active site of Zn-containing metalloenzymes, and large amounts of Zn are also found in exocrine and endocrine pancreas cells.[15] Zn is essential for cellular activities such as cellular division, DNA replication, and RNA and protein synthesis, as well as fatty acid metabolism.[16,17] Recent experiments suggest that oxygen-derived free radicals play an important role in the initiation and development of acute pancreatitis.[18,19] Cu, Zn-superoxide dismutase (SOD), Fe-SOD, and metallothionein, as a ligand of metal ions such as Cu and Zn, are considered to be free radical scavengers.[20,21] Ni also plays a role in metalloenzymes and is essential for the stability of DNA and RNA. Fe is present mostly in heme and non-heme iron proteins, and these proteins and enzymes play a major role in energy metabolism and oxidation. In view of the metabolic roles played by Zn, Ni, and Fe, their decrease in the early stage of acute pancreatitis would reduce either the polynucleotidic function and protein synthesis of the internal and external cell membranes or interfere with their lipid metabolism, or both. Furthermore, it appears that lack of these elements may reduce the induction of metallothionein,[22] Cu, Zn-SOD, and Fe-SOD. In this study, we found that acute pancreatitis was associated with a rise in serum Zn and P levels, but that this occurred in the group treated with low-dose cerulein. The exocrine pancreas could become resistant to the stimulation of a supramaximal dose of cerulein (100 μg/kg) compared with the dose of 50 μg/kg. Previous studies have shown that, at this point, acinar cells gradually became resistant to cholecystokinin (CCK) stimulation after the onset of acute pancreatitis in several different animal models.[23] Moreover, serum amylase level was not significantly different in the two acute pancreatitis groups, given low-dose and supramaximal doses of cerulein, in this study. This situation reflects an interesting state of hormone resistance that needs to be characterized by further experimental studies.

An elevation of serum Zn has been observed in chronic pancreatitis, after pancreatectomy, and after pancreatic duct ligation,[10,23] and human subjects with pancreatic insufficiency were found to have decreased absorption of serum Zn.[24] In our present study, increased serum Zn and Fe, and decreased pancreatic Zn and Fe were observed, compared with controls. A mechanism consistent with these findings and the literature reports would be one in which the absorption of Zn and Fe was decreased with acute pancreatitis. Another possibility is increased elimination of Zn and Fe from the pancreas. This is unlikely, however, since Zn is eliminated mainly via pancreatic juice, which contains a higher concentration of Zn than serum. We could find no reports of Fe homeostasis after the onset of acute pancreatitis. It is important to discuss other possible explanations for our results.

Ca plays a pivotal role in enzyme secretion. A significant increase of pancreatic Ca may result from an increase in Ca influx into the cell. Excess Ca in the pancreas may contribute to the initiation of the proteolytic cascade. Serum Ca levels are reduced with severe acute pancreatitis. The serum Ca level was not significantly altered in this model of mild acute pancreatitis. Acute pancreatitis induced by cerulein is thought to be a non-lethal and reproducible model.

Ti may be derived from the soil, and its Clarke's number (0.46) indicates that it is the tenth most abundant metal on the surface of the earth. However it is not clear why Ti accumulated in the pancreas, and its physiological function also remains unclear.

We observed that S was abundant in normal pancreatic tissue, and small amounts of S are found elsewhere in the human body. One possible explanation could be the presence in the pancreas of amino acids and/or enzymes containing S. It is known that metallothionein and Fe-containing enzymes also contain large amounts of S.

We conclude that the determination of metallic and other elements in the pancreas, using X-ray fluorescence, is useful for investigating the pathophysiology of cerulein-induced acute pancreatitis. This study suggests that a decrease of Zn and other elements in the pancreatic tissue could play an important role in acute pancreatitis induced by cerulein.

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