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Zinc Supplement Modulates Oxidative Stress and Antioxidant Values in Rats with Severe Acute Pancreatitis

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Abstract Oxidative stress is a main factor in the pathogenesis of severe acute pancreatitis (SAP). The ability of zinc (Zn) to retard oxidative processes has been recognized for many years. This study aims to examine the levels of free oxygen radicals and antioxidant enzyme in SAP rats and know the effect of Zn supplementation on free oxygen radicals and antioxidant system in rats with SAP. Forty-five male Wistar rats were divided into three groups—the SAP group (n=15), the Zn-treated group (n=15), and the controlled group (n=15). For the SAP group, sodium taurocholate is injected into the pancreatic duct to induce SAP; for the Zn-treated group, Zn (5 mg/kg) is subcutaneously injected immediately after injection of 5 % sodium taurocholate. Firstly, the activity of erythrocyte glutathione peroxidase (GSH-Px), erythrocyte superoxide dismutase (SOD), and the content of plasma malondialdehyde (MDA), which are the toxic products of oxidative stress, is measured. Secondly, the levels of free oxygen radicals in the liver and kidney are detected. The result showed that the activity of GSH-Px and SOD was lower in the SAP group than that in the controlled group, although the content of plasma MDA increased. However, the activity of SOD and GSH-Px in the Zn-treated group was not significantly decreased after comparing with the controlled group; in the mean time, the content of MDA was not significantly increased either. Moreover, the content of free radical in liver and kidney was higher in the SAP group compared with the controlled group, but the content of free radical in the Zn-treated group was not higher than that in the controlled group (p>0.05). All of the above indicated that Zn may recover the activity of free radical-scavenging enzymes and decrease the content of free radical for the SAP group rats. In conclusion, the content of free radical increase may be one of the reasons that SAP rats are injured, and it is possible for Zn to be used to treat SAP through scavenging free radical and increasing the activity of SOD and GSH-Px of erythrocyte.

Keywords Antioxidant enzyme · Free radical · Pancreatitis

Introduction

Usually, acute pancreatitis is a mild and self-limiting disease, but, in minority of cases, it develops into a severe disease with high morbidity and mortality [1]. Biphasically, there are two causes that result in death from severe acute pancreatitis (SAP). Early death is attributed to acute consequences of the pancreatic inflammatory process and the systematic inflammatory response with subsequent multiorgan dysfunction. The patients with severe acute pancreatitis may further contract with systemic inflammatory response syndrome (SIRS) causing damage to remote organs and ultimately multiple organs failure [2]. Late death is mainly caused by sepsis, especially infected pancreatic necrosis [3].

Metal ions are required active components of several proteins, including pancreatic enzymes [4]. The metals such as Zn, copper, chromium, selenium, and manganese have been found to be essential for normal biologic functions and now are termed as essential trace elements [5, 6], which play important role in the etiopathogenesis of acute pancreatitis.

It is well-known that Zn plays essential role in almost all aspects of metabolism. Its functions include structural and

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catalytic roles in metalloenzymes and other metalloproteins, as well as regulatory roles in such diverse processes as synaptic signaling and gene expression [7]. Besides, Zn plays an essential biochemical role that retards the oxidative processes, and it also serves as a potential antioxidant [8]. An abnormal Zn metabolism is accompanied with severe oxidative stress as a result of an increase in oxygen free radical production [8]. Long ago, Zn has been recognized as essential for the activity of a wide range of enzymes including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) [9]. SOD is one of the most important antioxidants [10]. GSH-Px is an abundant intracellular thiol that plays an essential role in the detoxification of oxidants, and acts as a critically important antioxidant in many different cell types [11].

As what we have demonstrated, in our previous study, the levels of Zn, copper, chromium, and manganese in the SAP group were lower than that in the controlled group [12]. The present study investigated the effect of Zn supplement on the levels of free oxygen radicals and antioxidant enzyme in rats with severe acute pancreatitis.

Methods and Materials

Animals and Study Groups

Forty-five pathogen-free male Wistar rats weighing 200–300 g and aging 9 to 11 weeks were provided by Laboratory Animal Center of Anhui Medical University of China. Animals were fasted overnight except with free access to water. All studies were performed in accordance with the guidance of the Committee on Care and Use of Laboratory Animals.

Forty-five male Wistar rats were divided randomly into three groups which serve as the controlled, SAP, and Zn-treated groups, respectively. The number of each group is 15. All the rats were then anesthetized with 2.5 % pentobarbital (0.1 mL/100 g body weight intraperitoneally). A midline laparotomy was performed, followed by ligation of the bile-pancreatic ducts close to the liver and duodenum. For the SAP and Zn-treated group, the pancreatic duct was retrogradely injected with 5 % sodium taurocholate (0.1 mL/100 g body weight) for 1 min and was stagnant for 4 min [13]. The Zn-treated group rats were then treated with Zn (Zn sulfate) via subcutaneous injection (5 mg/kg) immediately after 5 % of sodium taurocholate was injected. For the controlled group, sham operation was performed with the injection of normal saline into the pancreatic duct (without Zn administered). The abdomen was then closed. Twenty-four hours later, those rats were operated again, and blood samples were collected aseptically from the abdominal aorta.

Serum Amylase Activity and Histology

Serum amylase activity was measured by a chromogenic method with the Phadebas® Amylase Test. The pancreas were then rapidly removed and placed in 10 % neutral phosphate-buffered formalin for histological study.

The specimen was embedded in 10 % formaldehyde, stained with hematoxylin-eosin, and evaluated under optic microscopy. Pancreas tissue samples were examined by a pathologist, who was throughout unaware of the source of the specimens. Pancreatitis was confirmed by measuring amylase levels before and after the experiment and by histological examination.

Determination of the Free Radical Content of the Liver and Kidney Tissue

The ER 200D-SRC electron spin resonance (ESR) instrument, from the Bruker Company of Karlsruhe, Germany, was utilized. The temperature was set to 100 K. The liver and kidney samples of the controlled and experimental animals were homogenized in 10.0 mM Tris buffer (pH 7.5), and the homogenates were then centrifuged at 10,000×*g* for 1 min at 4 °C. Four-hundred-microliter aliquots of extracts were transferred into a test tube, and DMPO was added into a final concentration of 100 mM. Those actions' mixture was then transferred to a flat cell for ESR measurement [14]. Subsequently, the ESR waves were recorded [15]. The ESR settings and experimental conditions are as follows: microwave frequency, 9.46 GHz; microwave power, 10 dBmW; modulation, 2.5 Gpp; scanning time, 100 s; center, (3,350± 300)G magnetic, and accumulated, four times.

Determination of MDA, SOD, and GSH-Px Content

Blood samples collected from all study subjects were put into VENOJECT® tubes with EDTA (0.47 mol/L K_3-EDTA) between 1,000 and 1,800. All individuals were placed in a reclining position for a minimum of 10 min before blood sampling by the same phlebotomist. Within 4 h after sampling, the blood was centrifuged at 1,000×*g* for 10 min to separate the plasma. The buffy coat was removed, and the remaining erythrocytes were drawn from the bottom, washed three times in cold saline (9.0 g/L NaCl), and hemolyzed by adding the same weight of ice-cold demineralized ultrapure (MilliQ plus reagent grade; Millipore, Bedford, MA, USA) water to yield 50 % hemolysate. The hemolysates were frozen in 500-μL aliquots at −80 °C for later analysis.

The activity of GSH-Px and SOD was determined by modified Hafeman method and adjacent benzene three phenolic autoxidation method, respectively. Moreover, plasma MDA content was measured by thiobarbituric acid colorimetric method.

Statistical Analysis

All results were expressed as means ± standard deviation (SD). Data were analyzed using the SPSS statistical program (version 17.0 software, SPSS). The *T* test method was used to test their differences ($p<0.05$) which was considered as significant in statistics.

Results

Serum Amylase Detection

The level of serum amylase was increased, which confirmed the diagnosis of acute pancreatitis. Table 1 shows the values of amylase. The SAP group had higher level of amylase when compared with the controlled group. According to the statistical analysis, there was a significant difference between the SAP and the controlled groups ($p<0.05$; Table 1).

Pathological Examination

Furthermore, the findings of the histopathological analysis showed interstitial edema, parenchyma hemorrhage and necrosis, and inflammatory infiltration of neutrophils into the pancreatic tissue (Figs. 1 and 2).

SAP manifested with a rise in serum amylase activity and morphological evidence. In all animals, a marked elevation of serum amylase levels was observed for 24 h after sodium taurocholate infusion. The morphological changes were observed after sodium taurocholate infusion including interstitial edema, neutrophils infiltration, and necrosis change.

SOD Activity, GSH-Px Activity, and MDA Content

Comparing with the controlled group, erythrocyte SOD and GSH-Px activity of the SAP rats decreased, and plasma MDA content increased obviously; the difference is significant ($p<0.05$). Furthermore, erythrocyte SOD, GSH-Px activity, and plasma MDA content of the Zn-treated group had no significant difference compared with the controlled group ($p>0.05$). The results show that the antioxidative system is abnormal in SAP rats. However, Zn supplement can maintain normal antioxidant enzymes system (Table 2).

Table 1 Serum amylase of SAP rats ($n=15$ for each group, $\bar{x}\pm s$, time= 24 h)

	n	Amylase (U/L)
Groups		
Control	15	973±137
SAP	15	1,997±217*

*$p<0.01$ versus control

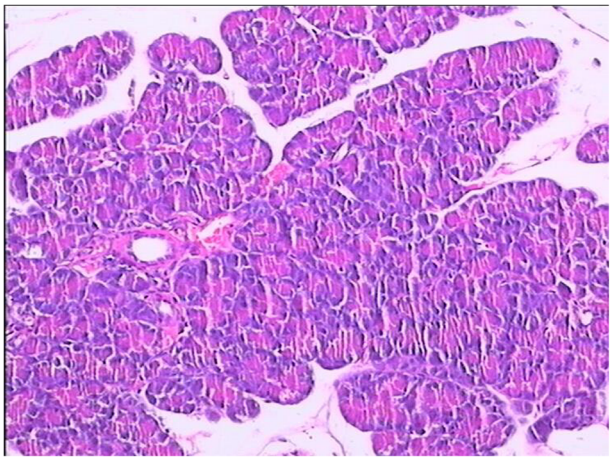

Fig. 1 The pancreatic tissue of the control group (HE ×100)

The Free Radical Content in Liver and Kidney Tissue

The free radical content in the liver and kidney tissue for SAP rats increased significantly compared with the controlled group. However, the free radical content in the liver and kidney tissue for Zn-supplemented group had no significant difference compared with the controlled group ($p>0.05$). The results show that Zn has antagonistic action on free radical changes in the liver and kidney tissue of SAP rats (Table 3, Figs. 3 and 4).

Discussion

SAP is an acute abdominal ailment with a high mortality rate, characterized by local inflammation and necrosis of the pancreatic tissue, but frequently affects extrapancreatic tissues leading to SIRS and other complications [16].

Oxygen-derived free radicals have been reported to play an important role in the pathogenesis of severe acute pancreatitis

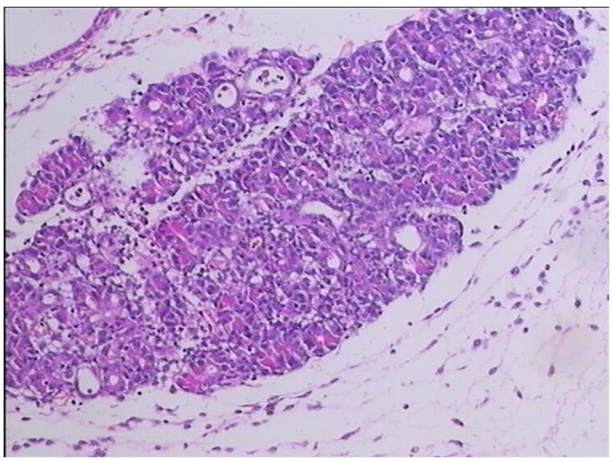

Fig. 2 Interstitial edema, neutrophils infiltration, and necrosis change in SAP group (24 h) (HE ×100)

Table 2 Erythrocyte SOD, GSH-Px activity, and plasma MDA content ($\bar{x}$ ±s)

Group	*n*	SOD activity (IU/g)	GSH-Px activity (IU/g)	MDA content (μmol/g)
Control	15	19.79±2.88	6.72±1.41	8.36±2.99
SAP	15	15.20±2.03*	3.91±1.64*	15.46±3.24**
Zn-treated	15	20.62±4.57***	7.51±2.09***	9.31±3.17***

*$p<0.05$ and **$p<0.01$ versus control; ***$p>0.05$ versus control

[17]. The normal cellular metabolism produces highly reactive free radicals—superoxide anion (O2−), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\cdot}$). These radicals are generally eliminated by antioxidant enzymes such as SOD, catalase, and GSH-Px. If the production of oxygen radicals exceeds the scavenging capacity of these enzymes, oxidative stress will develop. The excessive free radicals generated in the body during SAP may cause the accumulation of MDA, a lipid-oxidative product [18].

Zn is essential for cellular activities such as cellular division, DNA replication, and RNA and protein synthesis, as well as fatty acid metabolism. Zn supplementation seems to increase the survival of mice with acute pancreatitis [19]. It may play an important role in the pathophysiology of acute pancreatitis. Cu, Zn-SOD, and metallothionein, as ligand of metal ions such as Cu and Zn, are considered as free radical scavengers. In the previous study, the level of Zn, copper, chromium, and manganese in the SAP group was found to be lower than that in the controlled group [12]. The findings implicate Zn metabolism in the pancreas in the etiology and pathology of severe acute pancreatitis.

Antioxidant enzyme system inherent in the cellular defense system is the most important defense mechanism against reactive oxygen species (ROS) [20, 21]. GSH-Px and SOD act as antioxidants and have preventive effect against extensive production of ROS by severe acute pancreatitis [22]. In the current study, SOD and GSH-Px values were decreased by severe acute pancreatitis, although the enzyme activities were increased by Zn supplementation. These results are supported by a study that reported on the supplementation of Zn-protected cells exposed to pancreatitis-induced oxidative stress in the blood of human, possibly by affecting the lifetime of the ROS [23].

Table 3 Free radical content in liver and kidney tissue ($\bar{x}$±s)

Group	*n*	G factor	Liver (relative unit)	Kidney (relative unit)
Control	15	2.0036	43.0±2.83	44.3±3.78
SAP	15	2.0041	65.5±2.12*	68.5±3.91*
Zn-treated	15	2.0043	45.3±2.19**	46.3±2.28**

*$p<0.01$ versus control; **$p>0.05$ versus control

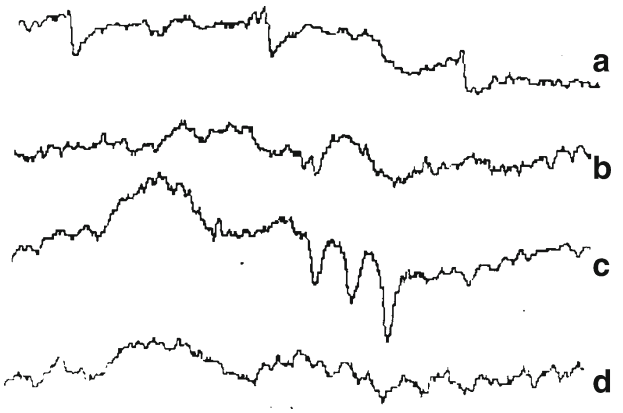

Fig. 3 Liver free radical spectrometer ($n=15$ for each group). *A* The ESR spectrum of manganese standard. *B* The ESR spectrum of control group. *C* The ESR spectrum of SAP group. *D* The ESR spectrum of Zn-treated group

During the present work, erythrocyte SOD and GSH-Px activity of the SAP rats were detected. The results show that SOD, GSH-Px activity, and plasma MDA content increased obviously compared with the controlled group; the difference is significant ($p<0.05$). The results state clearly that there is disequilibrium between free oxygen radical and antioxidant enzyme. In our study, we also find that Zn supplementation could reverse the disequilibrium.

Oxygen-derived free radicals have been implicated in the pathogenesis of acute pancreatitis. However, the involvement of free radicals in the extrapancreatic manifestations is not clear. Reactive oxygen metabolites have been found to play a role in the development of lung injury in acute pancreatitis [24]. However, so far, we are aware that data are seldom concerning free radical production in remote organs during the severe acute pancreatitis phase.

The current study demonstrates that the development of acute pancreatitis is associated with the generation of free radicals not only in the pancreas, but also in the liver and kidney. The free radical content in the SAP rats' liver and

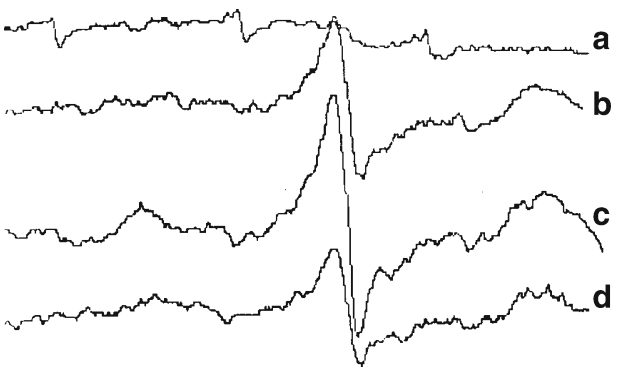

Fig. 4 Kidney free radical spectrometer ($n=15$ for each group). *A* The ESR spectrum of manganese standard. *B* The ESR spectrum of control group. *C* The ESR spectrum of SAP group. *D* The ESR spectrum of Zn-treated group

kidney is higher than that in the controlled group. However, the content decreased in the Zn-treated group. The results of the present study show that Zn supplementation is beneficial to the balance between free radical content and antioxidant enzyme systems in rats with severe acute pancreatitis as well to extrapancreatic organs such as the liver and kidney. Thus, the liver and kidney displayed almost as much evidence of oxidative stress as did the pancreas in the SAP rats.

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