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Relationships among Iron, Protein Oxidation and Lipid Peroxidation Levels in Rats with Alcohol-induced Acute Pancreatitis

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Abstract It has been previously shown that alcohol induces the damage of pancreatic parenchyma tissue, but the mechanism of this damage is still poorly understood. Assuming that oxygen radical damage may be the involved, we measured markers of oxidative damage in pancreatic tissue, blood serum, plasma, and whole blood of rats with early-stage alcohol-induced acute pancreatitis. Thirty-eight male Wistar rats were divided into three groups: the control group (group 1), the acute pancreatitis group 1 day (group 2), and 3 days (group 3) after the injection of ethyl alcohol into the common biliary duct, respectively. The levels of Fe in tissue and serum, whole blood viscosity, plasma viscosity, fibrinogen and homocysteine (Hcy) levels, erythrocyte and plasma malondialdehyde (MDA), and tissue and plasma protein carbonyl levels were found to be significantly higher in groups 2 and 3 than in group 1. However, the levels of reduced glutathione (GSH) in tissue and erythrocytes were significantly lower in groups 2 and 3 than in group 1. These results suggest that elevated Fe levels in serum and pancreatic tissue in rats with early-stage alcohol-induced acute pancreatitis is associated with various hemorheological changes and with oxidative damage of the pancreas.

Keywords Iron · Homocysteine · Malondialdehyde · Protein carbonyls · Viscosity · Acute pancreatitis

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Introduction

Alcohol abuse and dependence are the major cause of morbidity and mortality in various developed and developing countries. It has been known that excessive alcohol consumption is associated with increased risk of many diseases, including pancreatitis, which is an inflammatory disease [1]. It was reported in an animal study that the mortality from alcohol-induced pancreatitis was caused by a transient increase of pancreatic amylase output and plasma cholecystokinin (CCK) levels [2]. As ethanol is initially metabolized by the microsomal ethanol oxidizing system, creating an oxidative environment, oxidative stress appears to be the initial factor in the pathogenesis of alcoholic pancreatitis [1–4]. However, the detailed mechanism of induction of alcoholic pancreatitis is still not well understood.

Trace elements comprise a large number of elements ranging from normal constituents of the diet to environmental pollution and occupational hazards. Biological systems require a number of trace elements for their functioning. They exert profound influence on human health and disease states [5]. From these elements, iron (Fe) is essential for cellular functions, but in excessive amounts is toxic to cells. Redox-active iron ("free" or "labile" iron) is the optimal catalyst in such reactions and plays a decisive role in the generation of a variety of reactive oxygen species (ROS) [6, 7]. Several experimental studies have sought to link various measures of trace elements with the incidence of pancreatitis after the suggestion that the development of disease is related to iron stores [8–10]. However, these data are equivocal with positive associations detected in some studies but not others. Over the past years, in addition to the oxidant role of Fe, the toxic effects of Fe together with oxidative damage markers in alcohol-induced pancreatitis have been explored by limited studies [8, 10]. In addition, numerous studies were carried out to understand acute pancreatitis, but the Fe and oxidative stress markers related to alcohol consumption, which molecular mechanisms involved in the initiation and progression of pancreatitis, still remain to be investigated in greater detail.

According to our knowledge based on our searches of the literature, redox-active transition metals such as iron levels have not been determined previously in serum and pancreatic tissue in the early-stage alcohol-induced acute pancreatitis. In this experimental study, the changes in the Fe concentrations in the serum and pancreatic tissue of rats with acute pancreatitis rats were determined to show whether changes of the iron levels play a role in the induction of oxidative effects on lipids, proteins, and hemorheological parameters during the early-phase alcohol-induced acute pancreatitis (at 24 and 72 h). We also looked for a possible relationship between the Fe levels and other markers of oxidative damage such as blood viscosity, fibrinogen, hematocrit, and erythrocyte count.

Materials and Methods

The present study was approved by the Cerrahpasa Medical Faculty Laboratory Animals Ethics Committee, and all procedures with animals were performed in accordance with the guide of the Committee on Care and Use of Laboratory Animals (CCULA) [11]. Thirty-eight male Wistar Albino rats, weighing 200–280 g, were divided into three groups: the control group (group 1; $n=12$), acute pancreatitis groups at day 1 (group 2; $n=14$) and day 3 (group 3; $n=12$). The animals were fed on standard laboratory diet and water ad libitum before and after surgery. All animals were anesthetized with ether to undergo a midline laparotomy. Acute pancreatitis was induced by injection of 48% ethyl alcohol, in a

volume of 1 cm^3, into the common biliary duct using an insulin injector. The duct was transiently tied with a 1/0 silk suture to prevent retrograde leakage of alcohol. A sham laparotomy was performed in the control group. Groups 1 and 2 were killed 24 h later. Group 3 was also killed on postoperative day 3. Under ether anesthesia, 4 cm^3 (3–7 cm^3) of blood was taken by cardiac puncture, as well as taking out tissues.

For plasma and whole blood viscosity, malondialdehyde (MDA), reduced glutathione (GSH), homocysteine (Hcy), iron (Fe), and protein carbonyls, blood samples were collected into the vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) and without containing any anticoagulant. Fibrinogen was collected into the vacutainer tubes containing sodium oxalate. Plasma samples (containing EDTA) and serum (without anticoagulant) were obtained by centrifugation at 1,000×*g* for 20 min and stored at −70°C. Pancreatic tissue samples were homogenized after being frozen in −70°C deep freeze for biochemical parameters. Pancreatic tissue was immediately removed, weighed, and washed using chilled saline solution. Tissues were minced and homogenized (10% *w*/*v*) separately in ice-cold 1.15% KCl–0.01 M sodium, potassium phosphate buffer (pH 7.4) in a homogenizer. The homogenate was centrifuged at 10,000×*g* for 20 min at 4°C, and the resultant supernatant was used for GSH, MDA, and protein carbonyls. Preparation of erythrocytes were done with whole blood centrifugation for 5 min at 1,000×*g* and obtained after washing in 0.9% NaCl solution twice, and removed to measure.

All reagents, unless otherwise noted, were obtained from Sigma Chemicals (St. Louis, MO, USA).

Histological evaluation was performed according to Schoenberg et al. [12]. Tissue samples removed from the caput, corpus, and cauda of the pancreas were fixed in formalin for 24 h, paraffin embedded, and stained with hematoxylin and eosin (H&E). Light microscopy slides were examined and graded by a pathologist with experience in experimental pancreatitis who was unaware of the previous treatment.

The measurement of Fe levels in tissue samples were weighed and transferred into metal-free glass tubes for digestion. Tissues were kept at −20°C until use. The samples were first digested with 1 ml of concentrated nitric acid (69%) at 150°C in the furnace for 1 h and, 1 ml of perchloric acid (60%) was added to the cooled materials. The materials were then completely digested at 120°C until the materials diminished to the half of the original total volume. Digested materials were diluted with deionized water to 10 ml. The last dilutions of the samples were mixed on a shaker for 5 min just before measurement. The concentrations of Fe in pancreatic tissue and serum samples were measured by a flame atomic absorption spectrophotometer (Shimadzu AA-680, Tokyo, Japan) at 248.3 nm. Fe stock standard (of concentration 1,000 ppm) was obtained from Merck (Darmstadt, Germany). Results were calculated as micrograms per deciliter in serum and as micrograms per gram wet weight in tissue [13, 14].

The lipid peroxide levels were measured in erythrocyte, plasma, and supernatant obtained from tissue by using a thiobarbituric acid reactive substances (TBARS) assay, which monitors MDA production [15, 16]. The amount of MDA was calculated using an extinction coefficient (1.56×10^5 M^{-1} cm^{-1}). MDA concentrations were expressed as nanomoles per milliliter in plasma and as nanomoles per milligram of protein in tissue.

The concentrations of GSH in erythrocyte and supernatant obtained from tissue were determined in a modified optical test system [17]. In this system, GSH is oxidized by 5,5′-dithiobis-2 nitrobenzoic acid and then reduced by GSH reductase with NADPH as the hydrogen donor. The change of absorption was recorded at 412 nm on visible ultraviolet spectrophotometry. The GSH concentration was determined by using the molar absorption coefficient 13.6×10^{-4} M^{-1} cm^{-1}. GSH concentrations were expressed as

milligram per gram of hemoglobin in erythrocyte and as nanomoles per milligram of protein in tissue.

As carbonyl groups (aldehydes and ketones) may be introduced into proteins by ROS and free radicals, quantitation of protein carbonyls was performed by incubating equal volumes of the sample (plasma and supernatant obtained from tissue) and 2,4-dinitrophenylhydrazine (3.4 mg per 10 ml 1 M HCl) at 50°C for 1 h. After the reaction, proteins were precipitated with 20% trichloroacetic acid and the unreacted dye was removed by centrifugation. The pellet was dissolved in 1 M NaOH, and the absorbance at 450 nm was recorded. The molar absorbance coefficient (25,500 M^{-1} cm^{-1}) was used to calculate the carbonyl content [18]. Protein concentrations were determined by the Lowry method with bovine serum albumin as the standard [19]. The concentrations of protein carbonyls were expressed as nanomoles per milligram of protein in plasma and as nanomoles per milligram of protein in tissue.

Whole blood and plasma viscosity were made according to the recommendations of the International Committee for Standardization in Haematology (ICSH) using a Harkness capillary viscometer (Coulter Electronics, Ser. No. 6083, Luton, England) and evaluated in relation to distilled water (relative viscosity), the water bath of which was maintained at 37°C [20, 21]. Hematocrit was measured by microhematocrit centrifuge method. The hemoglobin content of blood samples was determined by the cyanmethemoglobin procedure [22]. Erythrocytes were counted on a Thoma hemocytometer, using a light microscope, at ×400 magnification. Fibrinogen was measured using the Sysmex CA-1500 autoanalyzer (Diamond Diagnostics, USA) with the Dade Behring kit. Concentrations of plasma total homocysteine were measured using Agilent 1100 Series HPLC (Agilent Technologies, USA) with Chromsystems diagnostics kit.

Statistical analyses were carried out with the SPSS 11.5 version program (SPSS, Chicago, IL, USA). Values were reported as the means±SD. The Mann–Whitney's *U* test, one-way analysis of variance, and Tukey's honestly significant differences test were used to evaluate the significance of differences in characteristics, Fe levels, MDA, GSH, protein carbonyls, viscosity, and blood parameters values between control and pancreatitis groups. Correlation analysis was performed by using Spearman's rank correlation coefficient. Differences were considered significant at $p<0.05$.

Results

During the early phase of alcohol-induced pancreatitis, which was studied on days 1 and 3 after injection, there was neither mortality nor body weight loss in the current study. There was no sign of acute pancreatitis in the control group (group 1). The pancreatic tissues of the rats in the control group were all normal, even on gross view. All rats in experimental group day 1 (group 2) and experimental group day 3 (group 3) developed acute pancreatitis. Inflammatory infiltration of neutrophils and mononuclear cells, interstitial edema, and focal necrotic areas were seen in the pancreatic tissue of the acute pancreatitis group. Grossly, all pancreatic tissues showed edematous swelling and necrotic areas.

The erythrocyte count and hematocrit were found to be decreased, whereas Hcy, fibrinogen, whole blood, and plasma viscosity were increased in group 3 compared to group 1. Hematocrit values were significantly lower in group 3 compared to group 2. In addition, hematocrit levels were decreased in group 2 than those in group 1. However, plasma viscosity was significantly higher in group 3 than those in group 2. These parameters levels were also significantly increased in group 2 than those in group 1 (Table 1).

Table 1 The Mean Erythrocyte Count, Hematocrit, Whole Blood Viscosity, Plasma Viscosity, Homocysteine, and Fibrinogen Levels in Control and Acute Pancreatitis (at 24 and 72 h) Groups

Parameters	Group 1 (control; $n=12$)	Group 2 (24 h AP; $n=14$)	p*	Group 3 (72 h AP; $n=12$)	p**	p***
Erythrocyte count (10^6/mm^3)	4.47±0.51	4.09±0.22	NS	3.95±0.20	<0.01	NS
Hematocrit (%)	42.25±3.57	38.28±2.16	<0.01	36.08±1.88	<0.001	<0.05
Whole blood viscosity (mPas)	2.74±0.27	3.16±0.24	<0.001	3.30±0.19	<0.001	NS
Plasma viscosity (mPas)	1.14±0.13	1.31±0.12	<0.01	1.44±0.14	<0.001	<0.05
Homocysteine (μmol/l)	2.59±0.45	3.38±0.49	<0.001	3.68±0.41	<0.001	NS
Fibrinogen (mg/dl)	141.08±16.40	235.00±35.24	<0.001	247.33±28.33	<0.001	NS

Results are expressed as the mean±SD.

AP: acute pancreatitis, *NS*: not significant

**p*, group 2 compared with group 1; ***p*, group 3 compared with group 1; ****p*, group 2 compared with group 3

The concentrations of Fe in serum and tissue were significantly increased in the pancreatitis groups compared to the control group. The levels of plasma protein carbonyls and tissue protein carbonyl, erythrocyte MDA, plasma, and tissue MDA levels were found to be increased in groups 3 and 2 than those in group 1. The levels of total protein and plasma protein carbonyl levels at day 3 acute pancreatitis were higher than those in the other groups. Furthermore, oxidative damage was shown to be associated with increased Fe levels in the acute pancreatitis groups. On the other hand, the levels of GSH in erythrocyte and tissue were lower in the acute pancreatitis groups than those in the controls (Table 2).

From Spearman's rank correlation analysis, we found a significant positive correlation between serum Fe levels and erythrocyte MDA in the experimental group day 1 of acute pancreatitis group ($r=0.695$, $p<0.01$). There were no correlations between Fe levels and erythrocyte count, hematocrit, Hcy, plasma viscosity, or whole blood viscosity. In the experimental group day 3 of acute pancreatitis (group 3), serum Fe levels were positively correlated in erythrocyte MDA ($r=0.849$, $p<0.01$). Tissue Fe levels were also weakly positively correlated with plasma viscosity ($r=0.644$, $p<0.05$). Moreover, serum Fe levels were positively correlated with plasma protein carbonyls ($r=0.705$, $p<0.05$). However, serum Fe levels were negatively correlated with tissue GSH ($r=-0.577$, $p<0.05$). There were no correlations between Fe levels and erythrocyte count, hematocrit, Hcy, or whole blood viscosity.

Discussion

Pancreatitis is an inflammatory process in the pancreas, and ROS have been implicated as an important factor in both the initiation and progression of acute pancreatitis [3, 23, 24]. Oxidative stress has also been recognized in association with all phases of pancreatitis, and oxidative stress markers have already been used for the possible diagnostic and prognostic value of measurements of parameters of oxidative damage in humans suffering from acute pancreatitis [7, 10, 12, 24]. It has been suggested that transition metals, such as iron, may

Table 2 The Levels of Fe, Total Protein, Protein Carbonyls, MDA, and GSH in Control and Acute Pancreatitis (at 24 and 72 h) Groups

Parameters	Group 1 (control; $n=12$)	Group 2 (24 h AP; $n=14$)	p*	Group 3 (72 h AP; $n=12$)	p**	p***
Serum Fe (μg/dl)	57.42±7.38	71.25±5.62	<0.001	75.18±6.47	<0.001	NS
Tissue Fe (μg/g wet weight)	46.29±6.35	59.18±7.73	<0.001	62.57±7.67	<0.001	NS
Total protein (g/dl)	6.30±0.28	6.23±0.28	NS	6.67±0.33	<0.01	<0.001
Plasma protein carbonyls (nmol/mg protein)	0.82±0.10	1.06±0.17	<0.001	1.24±0.13	<0.001	<0.01
Tissue protein carbonyls (nmol/mg protein)	0.98±0.14	1.49±0.20	<0.001	1.60±0.16	<0.001	NS
Plasma MDA (nmol/ml)	2.23±0.41	4.25±0.38	<0.001	4.42±0.37	<0.001	NS
Tissue MDA (nmol/mg protein)	3.96±0.45	9.17±1.45	<0.001	9.98±1.38	<0.001	NS
Erythrocyte MDA (nmol/g Hb)	1.43±0.22	3.36±0.27	<0.001	3.51±0.23	<0.001	NS
Erythrocyte GSH (mg/g Hb)	5.27±0.64	3.90±0.17	<0.001	3.74±0.13	<0.001	<0.05
Tissue GSH (nmol/mg protein)	1.38±0.27	1.12±0.22	<0.05	1.07±0.20	<0.01	NS

Results are expressed as the mean±SD.

AP: acute pancreatitis, *NS*: not significant

**p*, group 2 compared with group 1; ***p*, group 3 compared with group 1; ****p*, group 2 compared with group 3

participate in the oxidative damage of cells and tissues. However, the relationship between blood and tissue MDA and protein carbonyl levels and the concentrations of Fe in blood and tissue has not been thoroughly quantified in alcohol-induced acute pancreatitis. To our knowledge, this study is unique in examining the relationship between these oxidative damage markers and Fe status in the experimental alcohol-induced acute pancreatitis.

ROS have been implicated in the impairment of the pancreatic microcirculation in acute pancreatitis [3, 23, 24]. It is becoming apparent that metal-catalyzed oxidation-mediated protein oxidation is an early indicator of tissue damage and that the formation of protein carbonyl derivatives is associated with pathological conditions both in humans and in animal model systems [25]. As metal ions are an integral part of many proteins necessary for biological functioning, the role of redox-active transition metal ions in metal-catalyzed protein oxidation was intensively studied. The role of transition metals in oxidative damage has been implicated in many disorders [8–10]. Epidemiological evidence has raised concern that a moderate elevation in body iron stores may increase oxidative stress [8, 26]. Therefore, the iron–heart hypothesis, which is based on the premise that high body iron burdens lead to increased levels of redox-active iron and iron-mediated oxidative injury to the cardiovascular system, has continued to be hotly debated. Fe reacts avidly with ubiquitous, weak ROS such as superoxide anion and hydrogen peroxide to generate highly toxic hydroxyl radicals via Fenton/Haber-Weiss chemistry [26]. The main theoretical mechanism of protein carbonyl formation states that redox cycling cations such as Fe can bind to cation-binding locations on proteins and with the aid of further attack by hydrogen peroxide or superoxide anion can transform side-chain amine groups on several amino acids into carbonyls [26–30]. We showed that the concentrations of Fe in the serum and tissue were higher in acute pancreatitis groups compared to the control group. However,

Kashiwagi et al. [9] have shown that in the early stage of acute pancreatitis, the concentration of pancreas Fe was decreased, whereas the concentration of Fe in serum was increased in the pancreatic group. It has been showed that the levels of oxidative damage markers MDA and protein carbonyls are increased in acute pancreatitis [27, 31]. In the present study, we also found increased protein carbonyls and MDA in tissue and plasma in acute pancreatitis groups compared to the control group. These findings suggest that increased iron levels may contribute to products of oxidative damage in acute pancreatitis groups. One possible explanation for these finding may involve the products of oxidative damage inducing a change in protein–protein interactions in pancreatitis. MDA is known to cross-bind and induce secondary oxidative damage in plasma and tissue proteins [7]. From Spearman's rank correlation analysis, in the acute pancreatitis groups (groups 2 and 3), the increment of oxidative damage and iron levels in blood and pancreatic tissue were found and significant positive correlations were observed between Fe levels versus MDA and protein carbonyls, whereas there was a negative correlation between Fe and GSH levels. A common pathway in the pathogenesis of acute pancreatitis is the generation of ROS, and the most important defense mechanisms are free radicals scavengers, especially glutathione [17]. Depletion of glutathione has been described in all forms of acute pancreatitis. The present findings are in parallel with the literature data, and they strongly suggest that oxidative stress plays a central role in the pathophysiology of pancreatitis [31, 32, 33]. The question whether GSH in erythrocyte and tissue are able to prevent negative consequences of hyperhomocysteinemia is of interest as there are indications that the increased risk of some diseases posed by high total Hcy might be because of its prooxidant properties in the presence of transition metals. In the present study, we confirmed that Hcy levels in plasma were increased in the acute pancreatitis groups than in the control group. On the other hand, GSH levels in erythrocyte and tissue were decreased in the pancreatitis groups than in the controls. There were no significant correlation differences in measured Fe and Hcy in the acute pancreatitis groups and control group. It has been known that the hemodynamic and hemorheologic variables play an important role in the pathogenesis of pancreatitis. Plasma and whole blood viscosity are major factor to hemorheology [34]. Plasma viscosity is an indicator of blood flow in microcirculation. An elevated plasma viscosity indicates increased resistance to the flow in the level of tissue [35]. Jung et al. [36] reported that plasma viscosity mainly depends on plasma fibrinogen, albumin, globulin, and lipids levels. It has also been shown that cholesterol levels were increased in alcohol-induced acute pancreatitis [4]. Hematocrit and erythrocyte count determine the viscosity of whole blood. In the acute pancreatitis groups, we observed increased fibrinogen levels, whole blood viscosity, and plasma viscosity in comparison to the controls, whereas erythrocyte count and hematocrit were decreased in the acute pancreatitis groups compared to the controls. These findings parallel with the studies of Yan et al. [37] showing hemorheologic disturbance, which is one of the key factors in the pathogenesis of acute necrotizing pancreatitis. Meng et al. [38] investigated the mechanism of resveratrol in treating the underlying microcirculation disorder and lung injury after severe acute pancreatitis. We believe that decreased defense mechanism against oxidative stress, oxidative stress-induced impairment of the microcirculation, disruption of the microvascular integrity, decreased perfusion, increased capillary permeability, fluid transudation, hemorheological changes, and increased viscosity with elevated fibrinogen levels should be considered all together. In addition, it has been suggested that proteins modified directly by ROS and indirectly by reactive carbonyl compounds may cause viscosity changes in plasma [39]. It is likely that such biological reactions in the blood and pancreatic tissue would alter the physicochemical properties of proteins and lipids with resulting effects on the hemorheological changes.

It was agreed that antioxidant therapy would be used in acute pancreatitis, although contradictory observations have also been made. Selenium (sodium selenite), beta carotene, L-methionine, and vitamins C and E are already being used for the treatment of acute and chronic pancreatitis [40–42]. Therefore, a further complex study is desired to assess how antioxidant therapy may contribute to attenuate the redox metal ions changes and oxidative damage.

In conclusion, the data from our study demonstrate a significant positive relationship between Fe levels and MDA, and protein carbonyls levels and viscosity in the early phase of acute pancreatitis. These findings may indicate that elevated Fe levels synergizes with oxidative stress in causing alterations in the cellular changes found in the early stages of acute pancreatitis in rats to the various hemorheological parameters.

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References

1. Chowdhury P, Gupta P (2006) Pathophysiology of alcoholic pancreatitis: an overview. World J Gastroenterol 12(46):7421–7427
2. Saluja AK, Bhagat L (2003) Pathophysiology of alcohol-induced pancreatic injury. Pancreas 27:327–331
3. Andican G, Gelisgen R, Unal E, Tortum OB, Dervisoglu S, Karahasanoglu T et al (2005) Oxidative stress and nitric oxide in rats with alcohol-induced acute pancreatitis. World J Gastroenterol 11:2340–2345
4. Unal E, Uzun H, Kusaslan K, Dogan M, Genc H, Gunes P et al (2005) Serum paraoxonase (a high-density lipoprotein-associated lipophilic antioxidant) activity and lipid profile in experimental acute pancreatitis. Pancreas 31:84–87
5. Guntupalli JN, Padala S, Gummuluri AV, Muktineni RK, Byreddy SR et al (2007) Trace elemental analysis of normal, benign hypertrophic and cancerous tissues of the prostate gland using the particle-induced X-ray emission technique. Eur J Cancer Prev 16(2):108–115
6. Halliwell B, Gutteridge JMC (1992) Biologically relevant metal ion-dependent hydroxyl radical generation. An update. FEBS Lett 307:108–112
7. Weber H, Huhns S, Jonas L, Sparmann G, Bastian M, Schuff-Werner P (2007) Hydrogen peroxide-induced activation of defense mechanisms against oxidative stress in rat pancreatic acinar AR42J cells. Free Radic Biol Med 42(6):830–841
8. Herbert V, Shaw S, Jayatilleke E, Stopler-Kasdan T (1994) Most free-radical injury is iron-related: it is promoted by iron, hemin, holoferritin and vitamin C, and inhibited by desferoxamine and apoferritin. Stem Cells 12(3):289–303
9. Kashiwagi M, Akimoto H, Goto J, Aoki T (1995) Analysis of zinc and other elements in rat pancreas, with studies in acute pancreatitis. J Gastroenterol 30:84–89
10. Ilback NG, Benyamin G, Lindh U, Fohlman J, Friman G (2003) Trace element changes in the pancreas during viral infection in mice. Pancreas 26(2):190–196
11. Committee on Care and Use of Laboratory Animals (CCLA) (1985) Guide for the care and use of laboratory animal. Institute of Laboratory Animal Resources, National Research Council, Washington, DC, p 83
12. Schoenberg MH, Buchler M, Gaspar M, Stinner A, Younes M, Melzner I et al (1990) Oxygen free radicals in acute pancreatitis of the rat. Gut 31:1138–1143
13. Aydemir B, Kiziler AR, Onaran I, Alici B, Ozkara H, Akyolcu MC (2006) Impact of Cu and Fe concentrations on oxidative damage in male infertility. Biol Trace Elem Res 112(3):193–204
14. Karakoc Y, Yurdakos E, Gulyasar T, Mengi M, Barutcu UB (2003) Experimental stress-induced changes in trace element levels of various tissues in rats. J Trace Elem Exp Med 16:55–60
15. Stocks J, Dormandy TL (1971) The autoxidation of human red cell lipids induced by hydrogen peroxide. Br J Haematol 20:95–111
16. Buege JA, Aust STD (1978) Microsomal lipid peroxidation. Methods Enzymol 52:302–310

17. Anderson ME (1985) Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol 113:548–555
18. Levine RL, Wehr N, Williams JA, Stadtman ER, Shacter E (2000) Determination of carbonyl groups in oxidized proteins. Methods Mol Biol 99:15–24
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:269–275
20. International Committee for Standardization in Heamatology (ICSH) (1984) Recommendation for a selected method for the measurement of plasma viscosity. J Clin Pathol 37:1147–1152
21. Barutcu UB, Akyolcu MC, Toplan S, Karakoc Y, Kiziler AR, Oke N et al (1995) Effects of coronary artery diseases on haemorheological parameters and trace elements. J Basic Clin Physiol Pharmacol 6:289–294
22. Fairbanks VF, Klee GC (1986) Biochemical aspects of hematology. In: Tietz NW (ed) Textbook of clinical chemistry. Saunders, Philadelphia, PA, pp 1532–1534
23. Urunuela A, Sevillano S, de la Mano AM, Manso MA, Orfao A, de Dios I (2002) Time-course of oxygen free radical production in acinar cells during acute pancreatitis induced by pancreatic duct obstruction. Biochim Biophys Acta 15:159–164
24. Tsai K, Wang SS, Chen TS, Kong CW, Chang FY, Lee SD et al (1998) Oxidative stress: an important phenomenon with pathogenetic significance in the progression of acute pancreatitis. Gut 42:850–855
25. Stadtman ER, Oliver CN (1991) Metal-catalyzed oxidation of proteins. Physiological consequences. J Biol Chem 266(4):2005–2008
26. Kallianpur AR (2005) Iron and oxidative injury—a commentary on "Fatty acid-mediated iron translocation: a synergistic mechanism of oxidative injury" by D. Yao et al. Free Radic Biol Med 39:1305–1309
27. Winterbourn CC, Bonham MJ, Buss H, Abu-Zidan FM, Windsor JA (2003) Elevated protein carbonyls as plasma markers of oxidative stress in acute pancreatitis. Pancreatology 3:375–382
28. Biemond P, Van Eijk HG, Swaak AJG, Koster JF (1984) Iron mobilization from ferritin by superoxide derived from stimulated polymorphonuclear leukocytes, possible mechanism in inflammatory diseases. J Clin Invest 73:1576–1579
29. Gutteridge JMC (1986) Iron promoters of the Fenton reaction and lipid peroxidation can be releated from haemoglobin by peroxides. FEBS Lett 201:291–295
30. Kayali R, Cakatay U, Uzun H, Genc H (2007) Gender difference as regards myocardial protein oxidation in aged rats: male rats have increased oxidative protein damage. Biogerontology 8(6):653–661
31. Kriuchyna IeA (2000) Lipid peroxidation, the antioxidant system and the trace element level in acute pancreatitis. Likar Sprava 2:34–37
32. Frenzer A, Butler WJ, Norton ID, Wilson JS, Apte MV, Pirola RC, Ryan P, Roberts-Thomson IC (2002) Polymorphism in alcohol-metabolizing enzymes, glutathione *S*-transferases and apolipoprotein E and susceptibility to alcohol-induced cirrhosis and chronic pancreatitis. J Gastroenterol Hepatol 17(2):177–182
33. Freeman BA, Crapo JD (1982) Biology of disease. Free radicals and tissue injury. Lab Invest 47:412–426
34. Yarnell JWG, Baker IA, Sweetman PM, Bainton D, O'Brien JR, Whitehead PJ et al (1991) Fibrinogen, plasma viscosity and white blood cell count are major risk factors for ischemic heart disease. The Caephilly and Speedwell Heart Disease Studies. Circulation 83:836–844
35. Mchedlishvili G (1998) Disturbed blood flow structuring as critical factor of hemorheological disorders in microcirculation. Clin Hemorheol Microcirc 19:315–325
36. Jung F, Pindur O, Kiesewetter H (1992) Plasma viscosity dependence on proteins and lipoproteins: results of the Aachen study. Clin Hemorheol 12:557–571
37. Yan L, Lei Z, Cui X, Chen H, Yang Y, Li L et al (1993) The role of hemorheologic disturbance in experimental acute pancreatitis. Huaxi Yike Daxue Xuebao 24:71–74
38. Meng Y, Zhang M, Xu J, Liu XM, Ma QY (2005) Effect of resveratrol on microcirculation disorder and lung injury following severe acute pancreatitis in rats. World J Gastroenterol 11:433–435
39. Esterbauer H, Schaur RJ, Zollner H (1991) Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 11(1):81–128
40. Kuklinski B (1992) Acute pancreatitis—a "Free radical disease". Decreasing mortality by sodium selenite (Na_2SeO_3) therapy. Z Gesamte Inn Med 47(4):165–167
41. Kuklinski B, Buchner M, Müler T, Schweder R (1992) Anti-oxidative therapy of pancreatitis-an 18-month interim evaluation. Z Gesamte Inn Med 47(6):239–245
42. Kirk GR, White JS, McKie L, Stevenson M, Young I, Clements WD, Rowlands BJ (2006) Combined antioxidant therapy reduces pain and improves quality of life in chronic pancreatitis. J Gastrointest Surg 10(4):499–503