Acta Veterinaria (Beograd), Vol. 49. No.1, 29–38, 1999.

UDK 619:616.37.002:636.7

ALTERATIONS OF PANCREATIC ACINAR CELLS AND BIOCHEMICAL CHANGES IN SERUM DUE TO EXPERIMENTALLY INDUCED ACUTE PANCREATITIS IN DOGS

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(Received, 21. December 1998.)

Experimentally induced acute pancreatitis was initiated in 16 male German shepherd dogs by application of sodium taurocholate into the pancreatic duct system. Potential effects of oxygen free radicals were evaluated by premedication with an antioxidant: superoxide dismutase (SOD). Therefore in 8 animals sodium taurocholate solution was applied simultaneously with intra-arterial inoculation of superoxide dismutase, while another 8 dogs were treated only with sodium taurocholate. The effects of sodium taurocholate solution and SOD were evaluated biochemically bi the amylase activity, the concentrations of ceruloplasmin (Cp) and PAGE (polyacrylamide gel electrophoresis) of serum proteins, together with the pathohistological observations. The results confirmed that bile salt injected into the pancreatic duct caused widespread progressive necrotic hemorrahagic lesions in the acinar, ductal and vascular cells. The biochemical injury response in all examined dogs was hyperamylasemia, increased amount of macroglobulin and decreased albumin and globulin fractions. There was a statistically significant increase in amylase activity and statistically significant differences in Cp concentrations between the examined groups. Pathohistological alterations were similar in both experimental groups, except in their rate, which was rather sluggish in the group treated with SOD. We therefore assumed that the oxygen free radicals as well as the detergent effect of the applied bile salt mediated the initial lesions in the induction of acute pancreatitis.

Key words: acute pancreatitis, dog, oxygen free radicals, SOD, sodium taurocholate, ceruloplasmin; (Cp).

INTRODUCTION

The pathogenesis of acute pancreatitis is not completetly understood yet, despite numerous experimental models (Kueppers et al., 1993; Closa et al, 1993;

Foitzik et al, 1995; que et al, 1995; Celinski et al., 1995; Kaiser et al.1995). Recent experimental studies indicate that in different experimental models of pancreatitis many biochemical and morphological events are similar. These events include intracellular premature activation of trypsin, blockade of luminal enzyme secretion and appearance of intracellular vacuoles (Niederau et al., 1997). Also, recent findings suggested that oxygen-derived free radicals could play an important role in the development and progression of acute pancreatitis (Wang et al., 1996; Sendur et al., 1996; Zheng et al., 1994). These highly reactive metabolites are generated at an early stage of the disease, but the source and the stimulators of their enhanced production are still obscure. Treatment with antioxidants has been shown to reduce acinar cell injury and oedema in various animal models of pancreatitis, suggesting that the sustained generation of reactive oxygen species depletes celular antioxidant defences. The evidence for free radicals having an active part in developing acute pancreatitis conficts with some other studies (Schoenberg et al., 1995), suggesting that these agents might facilitate pancreatic dysfunction by enhancing pancreatic blood flow and secretion in response to their challenged production from endothelium and/or leukocytes, and/or macrophages (Sendur et al., 1996; Andersson et al., 1997). Thus there is clearly a need for well designed clinical trials to evaluate the protective role of antioxidant therapy in acute pancreatitis (Andersson et al., 1997).

In this paper, we intended to demonstrate the harmful role of free radicals and to find an optimal model for developing acute pancreatitis. Another purpose of this study was to examine the antioxidant effect of SOD. Therefore we initiated the disease by application of sodium-taurocholate into the pancreatic duct system. Having in mind its effect as radical scavenger (Closa et al., 1993), we investigated whether simultaneous intra-arterial application of superoxide dismutase (SOD) with bile salt solution may protect the acinar cells or not.

MATERIAL AND METHODS

Animals and experimental design: Sixteen, clinically healthy, male German shepherd dogs weighing between 10 and 30 kg were divided into two groups (each consisting of eight animals) and were laparotomized at definite time intervals. Before the surgical procedure the dogs were anesthetized with combelen (0.03 g/kg body weight).

Group 1. Eight animals received 20 ml of 10% sodium taurocholate solution into the pancreatic duct system and were sacrificed 5, 10, 20, 30 minutes and 4, 6, 8 and 48 hours after bile salt administration.

Group 2. Eight dogs were treated with sodium taurocholate, injected into the pancreatic duct, simultaneously with intra arterial (a. pancreatica duodenalis) application of SOD (8 mg Peroxinorm-Grunenthal, dissolved in 2 ml NaCl-18 mg). The animals were sacrificed after the bile salt and SOD administration, at the same time intervals as the previous group.

Group 3. Eight animals from the first group served as an autocontrol, for the study of biochemical parameters and the normal pancreatic structure, before application of sodium taurocholate.

Blood and pancreatic samples were collected at the above chosen time intervals.

Serum protein separation was explored by PAGE (7,5 polyacrylamide gel, TRIS glycine buffer system pH=8,3). The oxidase activity of Cp in serum was obtained using a colorimetric enzyme assay (Holmberg et al, 1951). Amylase activity was measured by a standard colorimetric method using a commercial test (Merck).

Processing of specimens for light microscopy: Pancreatic tissue samples were fixed in glutaraldehyde in 0.1 M phosphate buffer (pH 7,4). Samples were embedded in paraffin wax or Epon 812 (Fluka, Buchs, Switzerland). Paraffin wax sections were stained with hematoxylin and eosin (HE) and aldehyde fuchsin. Semi-thin sections were cut and stained with toluidine blue (1% aqueous solution).

RESULTS AND DISCUSSION

Numerous experimental models have been developed to study the pathogenesis of acute pancreatitis. The injection of bile salt solution into the pancreatic duct has been among the many stimuli used to initiate pancreatitis. The initiating factors of cell injury in the presented model are probably numerous: the detergent effect of sodium-taurocholate solution, the subsequent production of oxygen free radicals, probably by the activity of xanthine oxidase (Gutteridge et al., 1995), and the increase in intraductal pressure. All cited factors can damage the cell membranes and contribute to endothelial barrier malfunction.

The biochemical injury responses in the first group have been: hyperamylasemia (the results of amylase activity evaluation and the statistical data are given in Table 1.) decreased amount of albumin and globulin fractions and increased macroglobulin level (lipoproteins) (Figure 1). Statistical analysis of variance and the LSD-test clearly showed statistically significant differences in the serum α-amylase activity among the experimental groups. The highest amylase activity in the first experimental group was due to zymogen granule damage by oxygen free radical species and by the detergent effect of bile salt. An increased endothelial barrier permeability leads to leakage of serum proteins into the interstitium of the gland (Wang et al., 1995., Kueppers et al., 1993). In our opinion, lipoproteins enhanced the macroglobulin fraction, due to the disruption of cellular membranes.

In the second group of experimental animals, the activity of serum amylase was lower than in the first one, because of the SOD protection effect. The transferrin fraction was decreased, while the post transferrin fraction was elevated. This was in accordance with the findings of Oriordain et al., (1995). The amounts of albumin and globulin fraction were almost equal to those in control animals. In the region of post albumin, one more fraction appeared and we found increased levels of haptoglobins and macroglobulins in the serum of some dogs

(Figure 2). The elevition of macroglobulins indicates an acute phase response and the lipoprotein dissolution of membranes. Expecting that at least some of the animals can survive more than three days (as confirmed in our preliminary observations), we determined the concentration of ceruloplasmin. Although there was a statistically significant difference in Cp concentrations between the examined groups (Table 2), we could not impute this effect to the protective role of Cp as an acute phase protein. Despite the fact that ceruloplasmin rose to approximately double normal values on the third day after the prime insult we did not find increased concentrations in the intervals we have examined. Evidently, more investigations should be done to elucidate the role of Cp in dogs.

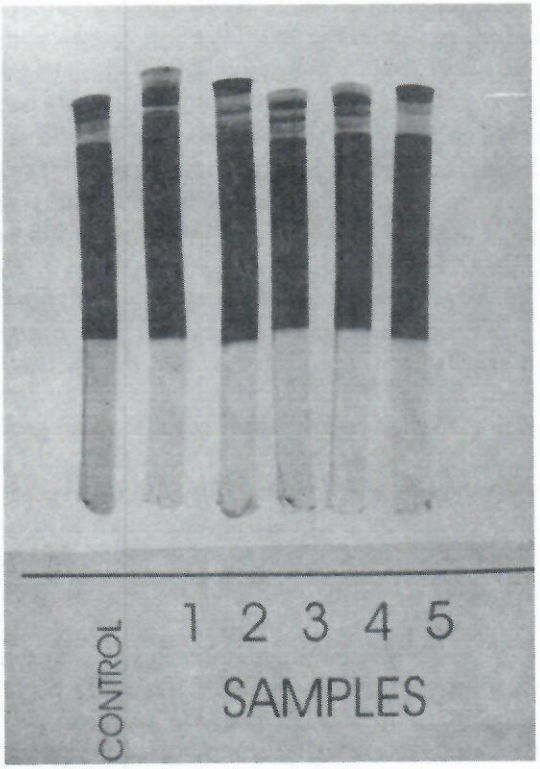

Figure 1. Electrophoresograms of the serum of dogs with acute pancreatitis and electrophoresogram of normal serum

Table 1. Serum α-amylase activity (IU/L) in experimental groups.

Group	n	$\bar{x}$	SD	SE
PT	8	1279.4	322.4	114.0
C	8	669.0	48.4	17.1
AP	8	2514.3	941.3	332.6
		$F = 21.365$; $DF = 2$ and 21; $p < 0.001$		
		LSD - test (PT:C) $p < 0.05$		
		(PT:AP) $p < 0.001$		
		(C:AP) $p < 0.001$		

PT – pretreatment with SOD
C – control
AP – acute pancreatitis

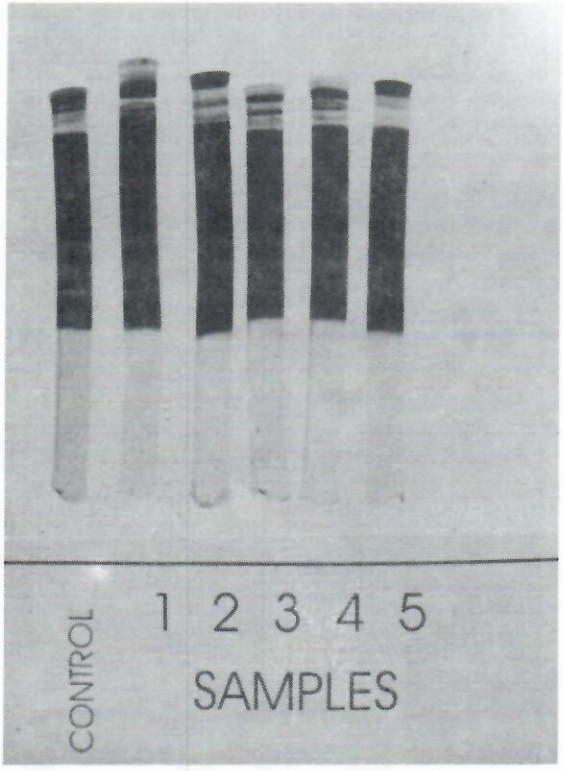

Figure 2. Electrophoresograms of the serum of dogs with acute pancreatitis pretreated with SOD

Table 2. Cp concentration in the sera of experimental groups and statistical data

Group	n	$\bar{x}$	SD	SE
PM	8	11.44	2.97	1.05
C	8	14.40	2.10	0.74
AP	8	17.01	2.78	0.98

$F = 8.900$; $DF = 2$ and 21; $p < 0.01$

LSD - test (PM:C) $p < 0.05$

(PM:AP) $p < 0.001$

PM – premedication
C – control
AP – acute pancreatitis

Figure 3. Dog pancreas interstitial acinar and cell oedema, HE, (x 100)

Considering pathohistological alterations we noticed that due to reinforced capillary permeability, pancreatic oedema developed during the first five minutes in all treated animals. It was followed by the dispersion of zymogen granules, and scattered acinar cell necrosis (Figure 4).

Figure 3 and 4

Pathohistological findings in the samples taken at subseqent time intervals, showed multiple necrosis of acinar cells and the migration of inflammatory cells such as macrophages and neutrophils.

In the last phase of acute pancreatitis, serious vascular lesions were noticed. The swelling of endothelial cells and their detachment with numerous erythrocytes in the necrotic parenchyma accompanied with fibrinoid degenera-

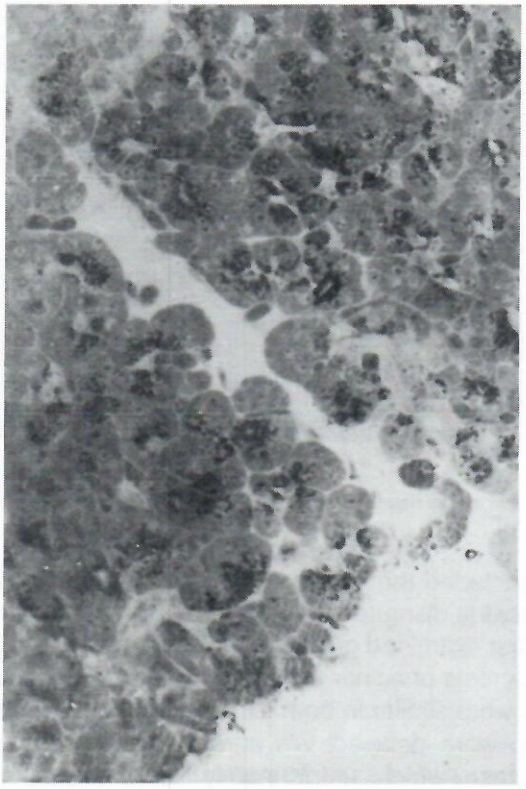

Figure 4. Dog pancreas dispersion of zymogen granules, scattered acinar cell necrosis, aldehyde fuchsin, semi-thin sections (x 400)

tion of capillary walls was observed. Vascular thrombosis caused ischaemia and contributes to the development of acute haemorrhagic pancreatitis with widespread necrotic lesions (Figure 5).

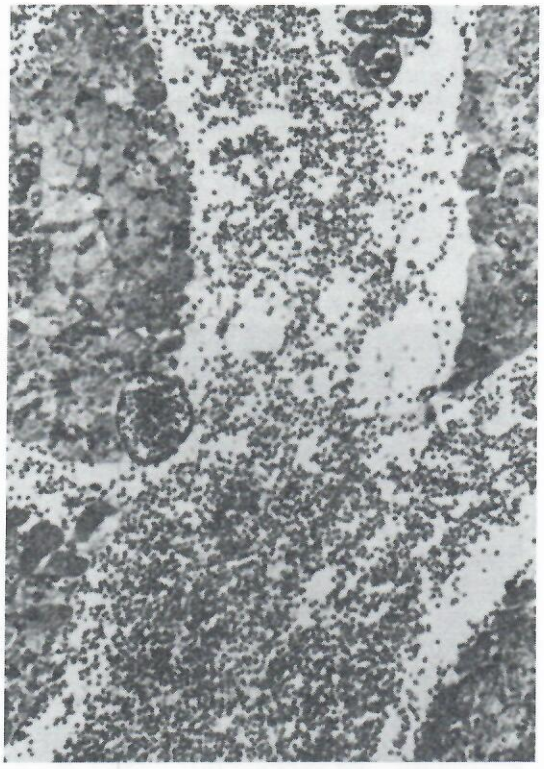

Figure 5. Dog pancreas, acute haemorrhagic necrosis and migration of inflammatory cells, HE, (x 200)

It can be concluded that in the early phase of pancreatitis in the animals treated with superoxide dismutase, swelling of pancreatic cells was less obvious compared to the first examined group. The dispersion of zymogen granules was also minor and necrosis of acinar cells was not so noticeable. All the pathohistological changes were similar in both experimental groups, but the changes in the second group were delayed. We attributed this to the protective role of superoxide dismutase, which, unfortunately has a very short half life. Consequently we concluded that oxygen free radicals mediate an important step in the

initiation of acute pancreatitis in the presented experimental model together with the detergent effect of the applied bile salt.

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ALTERACIJE PANKREASNIH ĆELIJA I BIOHEMIJSKE PROMENE U SERUMU NASTALE EKSPERIMENTALNO IZAZVANIM AKUTNIM PANKREATITISOM U PASA

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SADRŽAJ

Intraduktalnom aplikacijom natrijum tauriholata izazvan je eksperimentalni pankreatitis kod 16 pasa, rase nemački ovčar. Zaštitno dejstvo slobodnih radikala kiseonika ispitivano je intraarterijskom aplikacijom (a. pancreatica) rastvora superoksid dismutaze. Eksperimentalne životinje su bile podeljene u dve grupe. Osam pasa tretirano je rastvorom žučne soli, koji je aplikovan u sistem pankreasnih kanala, a osam životinja je istovremeno primalo rastvore natrijum tauriholata i superoksid dismutaze. Životinje iz prve eksperimentalne grupe, pre aplikacije rastvora žučne soli predstavljale su autokontrolu. Efekti aplikovanih rastvora određeni su biohemijskim i patohistološkim pretragama. U serumu pasa određivana je aktivnost amilaze i koncentracija ceruloplazmina, a frakcionisanje serumskih proteina rađeno je disk elektroforezom na poliakrilamidnom gelu. Rezultati ukazuju da je rastvor žučne soli aplikovan u pankreasni sistem kanala izazivao rasprostranjene hemoragične nekroze u acinarnim i endotelnim ćelijama kod obe grupe pasa. Biohemijske promene manifestovale su se hiperamilazemijom i povećanjem makroglobulinske frakcije, a smanjenjem količine albuminske i globulinske frakcije. Patološke promene bile su slične u obe ispitivane grupe s tim što su se promene sporije razvijale u grupi pasa tretiranih SOD-om. Zato smatramo da ova antioksidativna supstanca, uprkos svom kratkom poluživotu, ublažava efekat slobodnih radikala kiseonika koji imaju značajnu ulogu u otpočinjanju inicijalnih lezija u nastanku akutnog pankreatitisa, kao i deterdžentski efekat natrijum tauriholata.

Ključne reči: akutni pankreatitis, psi, slobodni radikali kiseonika, SOD, natrijum tauriholat, ceruloplazmin (Cp)