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Role of Reactive Oxygen Metabolites in Early Cardiopulmonary Changes of Acute Hemorrhagic Pancreatitis

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The role of reactive oxygen metabolites in extrapancreatic organ dysfunction associated with acute hemorrhagic pancreatitis was studied in dogs. Experimental pancreatitis was induced by the intraductal infusion of activated trypsin and taurocholate. Cardiac output, pulmonary and systemic blood pressure, pulmonary wedge pressure, central venous pressure, heart rate, blood gases and serum amylase were measured. Cardiac index, pulmonary and systemic vascular resistance, and the right and left stroke work were calculated. Systemic arterial and venous blood pressure and cardiac index gradually declined over 6 hr, while pulmonary mean blood pressure and pulmonary vascular resistance increased. Pretreatment of pancreatitis with catalase and superoxide dismutase prevented the rise in mean pulmonary blood pressure, moderated the rise in pulmonary vascular resistance, and decreased the rate and extent of the fall in cardiac index. These data suggest that reactive oxygen metabolites may play some role in the extraabdominal organ manifestations of acute pancreatitis.

KEY WORDS: reactive oxygen metabolites; progressive hypoxia; cardiopulmonary hemodynamics; extrapancreatic organ involvement.

Most patients with acute pancreatitis respond well to therapy. Patients that do poorly are usually those with extrapancreatic organ involvement, such as cardiovascular and pulmonary dysfunction (1). Extrapancreatic organ complications occur in 20% of patients with acute pancreatitis. Progressive hypoxia is a poor prognostic sign (2–5).

There is a great deal of evidence that implicates reactive oxygen metabolites in inflammatory, ischemic, and other disorders of the gastrointestinal system (6–9). In various canine preparations acute pancreatitis was significantly ameliorated by the use of enzymatic antioxidants (10). This suggests that reactive oxygen metabolites may be important in the pathogenesis of acute pancreatitis. The pathogenesis of the extrapancreatic organ involvement is unknown. The aim of this study was to determine the early cardiopulmonary changes of acute hemorrhagic pancreatitis in a canine model and the effect of pretreatment with catalase and superoxide dismutase on these cardiopulmonary changes.

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MATERIALS AND METHODS

Ten mongrel dogs weighing 15–20 kg were anesthetized with 30 mg/kg of sodium pentobarbital given intravenously. Dogs were intubated with an endotracheal tube and placed on a mechanical ventilator using room air. The tidal volumes were set at 10 cc/kg body weight with a

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TABLE 1. SEQUENTIAL CHANGES IN HEMODYNAMICS DURING DEVELOPING PANCREATITIS

Parameter*	Time (hr) 0	1	2	3	4	5	6
Systemic blood pressure (mm Hg)							
Systolic	101 ± 14	60 ± 14	73 ± 11	82 ± 10	85 ± 10	85 ± 10	77 ± 6
Diastolic	70 ± 16	23 ± 16	35 ± 26	51 ± 22	62 ± 11	52 ± 23	42 ± 25
Pulmonary blood pressure (mm Hg)							
Systolic	15 ± 3	15 ± 11	18 ± 11	24 ± 13	14 ± 4	23 ± 13	27 ± 16
Diastolic	6.6 ± 2.1	4.8 ± 2.0	5.6 ± 2.4	7.6 ± 3.5	7.2 ± 4.0	6.8 ± 4.2	5.7 ± 4.0
Pulmonary wedge pressure (mm Hg)	4.2 ± 2.1	2.7 ± 1.4	2.5 ± 1.9	2.4 ± 1.0	2.6 ± 1.6	3.0 ± 2.9	2.3 ± 1.9
Central venous pressure (mm Hg)	3.4 ± 1.0	2.0 ± 1.4	3.0 ± 1.2	3.0 ± 1.2	2.5 ± 1.6	3.2 ± 1.7	2.5 ± 1.8
Cardiac index (liters/min/m^2)	9.4 ± 3.0	6.1 ± 3.2	5.9 ± 2.9	5.2 ± 2.6	4.2 ± 1.7	3.7 ± 1.9	2.9 ± 2.4
Systemic vascular resistance (dyne-sec/cm^5-m^2)	654 ± 125	428 ± 179	598 ± 191	888 ± 213	1290 ± 270	1299 ± 325	1377 ± 330
Pulmonary vascular resistance (dyne-sec/cm^5-m^2)	43 ± 13	72 ± 37	99 ± 34	162 ± 59	130 ± 34	201 ± 62	284 ± 82

*Data are given as mean ± SD.

respiratory rate of 20 breaths per minute. No changes were made in the ventilator setting throughout the experiment. A Swan-Ganz thermodilution catheter was placed in the pulmonary artery via the femoral vein. The femoral artery was catheterized for systemic blood pressure monitoring and obtaining blood samples. Ringer's lactate, 2–2.5 liters, was given intravenously to maintain a urine output of 30 ml/hr in both groups. Electrocardiogram, systemic and pulmonary systolic and diastolic pressures, central venous pressure and body temperature were continuously recorded throughout the study. Cardiac output, pulmonary artery wedge pressure, arterial blood gases, and mixed venous gases were obtained on an hourly basis for 6 hr. Cardiac index, right and left ventricular stroke work, systemic vascular resistance, and pulmonary vascular resistance were calculated by the method of Walker and Taylor (11).

After laparotomy the duodenum was opened, and the major pancreatic duct opening was identified and cannulated with a 20-gauge catheter. Acute hemorrhagic pancreatitis was induced in the control (group I) and experimental (group II) animals by infusion of 250,000 units of benzoyl L-arginine ethyl ester hydrochloride (BAEE) crystalline trypsin (type XIII, Sigma Chemical Company, St. Louis, Missouri) and 1.8 g of sodium taurocholate (Sigma) in 20 ml of Sorenson's buffer at a pressure of 30 cm of water over a 30-min period (12, 13). The abdomen was closed following duodenal closure, and zero time measurements were obtained at the end of infusion. In addition, group II was also treated with 50 mg of canine superoxide dismutase, 2500 units/mg (Sigma) and 50 mg canine catalase, 1000 units/mg (Sigma) in 100 ml of saline by intravenous infusion over a half-hour period prior to the induction of pancreatitis (10). The time interval between the completion of infusion of these agents and of trypsin and taurocholate was 8–10 min. Intravenous infusion of the enzymes that inhibit reactive oxygen metabolites was not continued after induction of pancreatitis. The lungs and pancreas were examined postmortem in all animals for the presence of edema, hemorrhage, or weight changes.

A *t*-test and two-way analysis of variance with interaction were used for statistical analysis and statistical differences were inferred at $P < 0.05$. Means and standard deviation of the data were calculated.

RESULTS

The hemodynamic data of the animals with untreated pancreatitis (group I) are summarized in Table 1. Systolic and diastolic systemic blood pressures fell by 41 and 67%, respectively, at the first hour after inducing pancreatitis ($P < 0.05$), and then remained below zero time values. Pulmonary mean blood pressure gradually rose over the 6-hr period ($P < 0.05$), while the wedge pressure decreased ($P < 0.05$). Following a fall of 41% at 1 hr ($P < 0.05$), the central venous pressure gradually rose again to levels below initial values. Cardiac index decreased by 35% ($P < 0.05$) and then declined slowly over the 6 hr at a rate of 0.67 liters/min/hr. The systemic vascular resistance fell by 35% at the first hour and then progressively increased and doubled in magnitude ($P < 0.05$), whereas the pulmonary vascular resistance rapidly rose by fivefold at a rate of 36 dyne-sec/cm^5-m^2/hr.

The hemodynamic data of the animals pretreated with catalase and superoxide dismutase before induction of pancreatitis are given in Table 2. Systemic systolic and diastolic blood pressures decreased by 21 and 47% at the first hour ($P < 0.05$), and then the levels fluctuated. Pulmonary mean blood pressure and wedge pressure decreased ($P < 0.05$). Although central venous pressure fell by 33% at the first hour ($P < 0.05$), the pressure gradually increased to exceed the initial pressure by 83% ($P < 0.05$). Cardiac index decreased by 12% in the first hour, then declined at a rate of 0.17 liters/min/hr for

TABLE 2. SEQUENTIAL CHANGES IN HEMODYNAMICS AFTER ENZYMATIC ANTIOXIDANT PRETREATMENT OF DEVELOPING PANCREATITIS

Parameter*	Time (hr)						
	0	1	2	3	4	5	6
Systemic blood pressure (mm Hg)							
Systolic	103 ± 10	73 ± 21	84 ± 20	78 ± 15	82 ± 15	74 ± 21	60 ± 18
Diastolic	68 ± 4	36 ± 4	50 ± 6	48 ± 16	52 ± 17	48 ± 17	34 ± 14
Pulmonary blood pressure (mm Hg)							
Systolic	17 ± 2	12 ± 3	15 ± 1	16 ± 3	16 ± 4	16 ± 3	13 ± 1
Diastolic	7.8 ± 0.7	5.2 ± 2.0	5.2 ± 1.5	7.2 ± 0.7	5.8 ± 2.4	6.2 ± 2.4	5.0 ± 0.8
Pulmonary wedge pressure (mm Hg)	4.6 ± 2.3	2.2 ± 1.9	2.5 ± 1.4	3.2 ± 1.7	2.9 ± 1.8	3.4 ± 2.2	3.0 ± 1.4
Central venous pressure (mm Hg)	3.0 ± 1.4	2.0 ± 1.4	2.6 ± 0.8	2.9 ± 1.0	3.2 ± 1.9	5.5 ± 0.9	5.3 ± 1.8
Cardiac index (liters/min/m^2)	9.3 ± 2.9	8.2 ± 2.5	8.0 ± 3.4	7.3 ± 3.5	7.3 ± 3.4	7.7 ± 3.5	5.0 ± 0.8
Systemic vascular resistance (dyne-sec/cm^5-m^2)	659 ± 111	451 ± 103	586 ± 178	605 ± 177	644 ± 192	531 ± 195	597 ± 189
Pulmonary vascular resistance (dyne-sec/cm^5-m^2)	52 ± 18	50 ± 13	59 ± 19	78 ± 20	69 ± 19	63 ± 22	75 ± 26

*Data are given as mean ± SD.

5 hr, and at the sixth hour fell by 32% ($P < 0.05$). Although the systemic vascular resistance fell at the first hour, it rose to near initial values. In contrast, the pulmonary vascular resistance gradually increased by 50% ($P < 0.05$), at a rate of 4 dyne-sec/cm^5-m^2/hr which was much less in group I ($P < 0.05$).

There was a gradual decline in the left and right ventricular stroke work in both groups. Arterial oxygen saturation fell by 20% in group I and 12% in group II, but the levels were not significantly different from each other. Initial arterial P_{CO_2} ranged from 31 to 35 Torr in both groups, and did not exceed 40 Torr throughout the experiment. There were no significant changes in P_{CO_2} or other blood gas parameters between the two groups.

The gross appearance of lungs was similar and showed no macroscopic pathology. The presence of acute hemorrhagic pancreatitis was confirmed on gross inspection of the pancreas and retroperitoneum. Serum amylase values exceeded 2000 Somogyi units/100 ml in all dogs at 6 hr.

DISCUSSION

There is ample evidence to suggest the role of reactive oxygen metabolites in the pathogenesis of acute pancreatitis (7, 15). Enzymatic antioxidants were found to have a protective effect against lung injury associated with α-naphthylthiourea (15). Since the half-life of superoxide dismutase is less than 6 min, superoxide dismutase and catalase were infused intravenously over 30 min prior to induction of pancreatitis to limit the effects of reactive oxygen metabolites in lungs. The amount of time that is required to actually cause pancreatic damage and the ensuing release of toxic substances which perturb extrapancreatic organs is not known. With our model the dogs were pretreated with superoxide dismutase and catalase to moderate the damage to the pancreas and perhaps reduce the release of these substances into the circulation. Since the biological half-life of these enzymatic antioxidants is short, these agents should not alter reactive oxygen metabolite formation in extrapancreatic organs.

After the induction of acute hemorrhagic pancreatitis, a dramatic deterioration of cardiovascular parameters occurred followed by stabilization at a subnormal level. Pretreatment with enzymatic antioxidants moderated the deterioration in the hemodynamic and pulmonary vascular parameters, but had no effect on arterial oxygenation, or the gross appearance of the lungs. It is possible that gas perfusion may have been modified by using positive pressure ventilation. Several clinical studies (2, 16–18) have shown that an early hypoxia occurred within initial 48 hr at the onset of acute pancreatitis. Cobo et al (16) found good arterial oxygen saturation in 33 patients with acute pancreatitis.

There was a progressive decline in cardiac index in both groups of dogs, but the rate of decline was less in animals treated with superoxide dismutase and catalase. Our study confirms other reports that cardiac output falls with pancreatitis (19, 20). An increase in cardiac index has been noted by others (21, 22).

Pulmonary hypertension has been observed in patients with acute pancreatitis and in dogs with experimental hemorrhagic pancreatitits (16, 23). Other investigators, however, have not found these

pulmonary changes (19, 24, 25). In our study pulmonary mean pressures and vascular resistance increased after induction of pancreatitis. The increase in pulmonary vascular resistance was significantly less in dogs treated with superoxide dismutase and catalase. This increase in pulmonary vascular resistance without a change in pulmonary pressures can be explained on the basis of a reduction in cardiac index.

Systemic vascular resistance also increased in the control group. This may be a reflection of either hypovolemia or the action of toxic oxygen metabolites on the heart. In the enzyme-pretreated group, the lack of a change in systemic vascular resistance may be related to a protective effect of enzymatic antioxidants on the heart. Although the increase in pulmonary vascular resistance may also be caused by either of these mechanisms, the response seen in pulmonary vascular resistance in the control group was much greater than the systemic response, suggesting an action of additional local pulmonary factors. Since the change in pulmonary vascular resistance was significantly less in dogs treated with superoxide dismutase and catalase, a possible role of reactive oxygen metabolites in the pathogenesis of pulmonary dysfunction is suggested.

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