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EFFECTS OF EXPERIMENTAL ACUTE PANCREATITIS IN DOGS ON METABOLISM OF LUNG SURFACTANT PHOSPHATIDYLCHOLINE

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SUMMARY: Acute haemorrhagic pancreatitis was produced in the dogs by transduodenal injection of autologous bile into the main pancreatic duct. There was no significant change in the activity of three regulatory enzymes of phosphatidylcholine biosynthesis (glycerophosphate acyltransferase, cytidyltransferase and cholinephosphotransferase) in lung; however, there was a 42% decrease in the amount of dipalmitoyl phosphatidylcholine (surfactant) in lung lavage due to acute pancreatitis. The decrease in lavage phospholipid content was associated with (a) 5-fold increase in phospholipase A_2 activity of lung lavage, and (b) massive accumulation of osmiophilic spheriod structures in the alveolar space.

Adult respiratory distress syndrome (ARDS) is a rapidly progressive disorder with a mortality rate of 50% or more and is associated with a variety of medical problems including acute haemorrhagic pancreatitis (1). The respiratory complications, such as atelectasis, pulmonary effusion, pulmonary hypertension, and pulmonary edema are the most common disorders seen in ARDS associated with acute pancreatitis (1-3). It has been suggested that the surfactant system in lung is damaged in ARDS(4). However, reports on surfactant in ARDS are scanty, and involve characterization of surface activity and phospholipids post mortem (5,6). Although these studies demonstrate evidence of surfactant deficiency, it may be due to terminal or post-mortem changes and thus not represent mechanisms that lead to respiratory failure.

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The breakdown of surfactant phosphatidylcholine due to an increase in the activity of phospholipase A_2 in lung lavage has been suggested to be one main aetiologic factor in the development of ARDS in pancreatitis (3,7,8). However, one can not overrule the possibility that a decrease in the ability of lung to synthesize phosphatidylcholine is responsible for the lowering of the amount of surfactant phosphatidylcholine in lung lavage. Another possibility is that in acute pancreatitis, there is no defect in the phospholipid biosynthesis, but there is a defect in the rearrangement of the osmiophilic spheriods to the tubular myelin structures in alveolar space and as a consequence, less surfactant phosphatidylcholine is released into the lung lavage. It should be noted that the accumulation of osmiophilic spheroids in the alveolar space has previously been demonstrated by us as a marker of lung toxicity (9), which might be a case in acute pancreatitis. In order to get an answer to these questions, we have developed here acute pancreatitis in dogs, and measured the activity of three regulatory enzymes (glycerophosphate acyltransferase, CTP: phosphocholine cytidyltransferase and cholinephosphotransferase), involved in phosphatidylcholine synthesis, as well as studied the ultrastructural characteristics of lung. This study was also designed to include experiments to confirm whether the decrease in the amount of surfactant phosphatidylcholine in acute pancreatitis is associated with the increase in phospholipase A_2 activity in lung lavage.

METHODS AND MATERIALS

Animals

Adult mongrel dogs (20-25kg) were anesthetized with sodium pentothal injection, maintained with 1% halothane and room air and ventilated endotracheally. Intrevenous normal saline was given at 10ml/kg/h per fluid maintenance. Acute haemorrhagic pancreatitis was produced in the dogs by transduodenal low-pressure injection of 0.5 ml/kg of autologous bile obtained from the gall bladder into the main pancreatic duct (3,8). The common

duct and accessory pancreatic duct was ligated. The development of pancreatitis was verified by first noting bile staining of the pancreas at the time of injection and finally by pathological evidence of acute haemorrhagic pancreatitis at the termination of the experiment after 12 h (8). Control dogs were similarly anesthetized, ventilated and subjected to laparotomy at which time bile was aspirated from the gallbladder, a duodenotomy was done and the pancreatic duct was isolated but not injected. In addition, the accessory pancreatic duct and common duct was not ligated. The control animals were also ventilated for 12 h and received the same volume of intravenous saline. At 12 hr, the dogs were sacrificed, and the lungs perfused with normal saline. A funnel was inserted into the pulmonary artery via the right ventricle and the left artery was cut to allow blood to wash out of the lung. During the perfusion, the lungs were ventilated endotracheally by a respirator. The lungs were removed and lavaged with three successive rinse of 500 ml of cold normal saline. The collected specimens were centrifuged at 800 g for 10 min, and the supernatant was used for the determination of the amount of phospholipids and protein and phospholipase A_2 activity. The lung specimens from control and experimental dogs were also collected for biochemical and ultrastructural studies.

Analysis of Lavage

Lipid was extracted from lung lavage fluid with chloroform: methanol and the amount of phospholipids and dipalmitoylphosphatidylcholine (surfactant) was determined as described elsewhere (10). The protein content of lavage fluid was determined by the procedure of Lowry et al. (11) and the phospholipase A_2 activity was assayed by the method of Zieve and Vogel (7).

Biochemical Assays

The lungs were washed in ice-cold saline, blotted dry, and weighed. The excised lungs were then minced with scissors and homogenized in four volumes of 0.25 M sucrose/1mM EDTA (pH7.4), in a Potter-Elvehjen homogenizer. The subcellular fractions (mitochondria, microsomes, and cytosol) were prepared as described by Stith and Das (12). Glycerophophate acyltransferase (13) and cholinephosphotransferase (12) activity was measured in both mitochondrial and microsomal fractions. The cytidyltransferase activity in the cytosol was measured according to Stern et al. (14).

Ultrastructural Analysis

Lung biopsies from control and experimental dogs were fixed with 2.5% glutaraldehyde in 0.1 M Sorensen's phosphate buffer, pH 7.2 for 4 h at 5°C (9). Tissue samples were washed for 1 h in buffer and treated for 1 h with 1% osmium tetroxide in 0.1 M Sorensen's phosphate buffer. The samples were then dehydrated in 50,70,95 and 100% ethanol, exchanged with propylene oxide and allowed to infiltrate overnight in 1:1 propylene oxide - Epon 812 mixture. The tissue was embedded in Epon 812. 1-μM thick sections were cut with a glass knife, stained with toluidine blue and surveyed by light microscopy. Thin sections were stained with uranyl acetate and lead citrate for ultrastructural analysis. A Philips EM 301 transmission microscope was used for electron microscopic examination.

RESULTS AND DISCUSSION

The transduodenal injection of bile caused severe interstitial haemorrhage and multifocal acinar infarctions in pancreas as well as vascular congestion and emphysema in lung (photographs not shown). There was no significant change in the activity of three regulatory enzymes of phosphatidylcholie biosynthesis (glycerophosphate acyltransferase, CTP: phosphocholine cytidyltransferase, and cholinephosphotransferase) in lung (Table 1); however there was a 42% decrease in the quantity of surfactant phospholipids in lung lavage (Table 2). This decrease in lung surfactant was associated with about five-fold increase in the activity of phospholipids A_2 in lung lavage (Table 2). Thus it is suggested that there is no change in the biosynthesis of surfactant in acute pancreatitis; but there is an increase in the breakdown of surfactant. It is important to note that in ARDS associated with hypovolemic shock, there is also no change in the biosynthesis of surfactant in lung (15).

Even though, the decrease of surfactant phospholipid has been associated with increased activity of phospholipase A_2, we

TABLE I

Effects of Acute Pancreatitis on Activity of Glycerophosphate Acyltransferase, Cytidyltransferase and Cholinephosphotransferase in Dog Lung

	Mitochondria		Microsomes		Cytosol	
	Control	Test	Control	Test	Control	Test
Glycerophosphate-Acyltransferase	1.5 ± 0.1	1.2 ± 0.2	2.4 ± 0.2	2.0 ± 0.2		
Cytidyltransferase					12.6 ± 1.3	11.9 ± 1.8
Cholinephospho-transferase	1.3 ± 0.1	1.5 ± 0.1	2.3 ± 0.2	2.1 ± 0.2		

Enzyme activity are expressed as n moles/min/mg protein. Values are the mean ± S.E. of 4 animals.

TABLE 2
Effect of Acute Pancreatitis on Phospholipid Composition and Phospholipase A_2 Activity of Lung Lavage in Dog

	Control	Test
Total phospholipids (mg/g of wet lavaged lung)	2.92 ± 0.31	1.52 ± 0.41
Dipalmitoyl Phosphatidylcholine (mg/g of wet lavaged lung)	1.62 ± 0.11	0.53 ± 0.12
Total Protein (mg/g of wet lavaged lung)	0.42 ± 0.02	0.44 ± 0.02
Phospholipase A_2 Activity	3.51 ± 0.31	18.52 ± 1.11

Phospholipase A_2 activity is expressed as n mol phosphatidylcholine hydrolyzed per mg protein. All values are mean ± S.E. of 4 animals.

do not know at this time what is the cellular origin of phospholipase A_2 and why its activity is increased. However, we have observed massive accumulation of osmiophilic inclusion bodies (osmiophilic spheriods, OS) in alveolar space (Fig. 1). Such

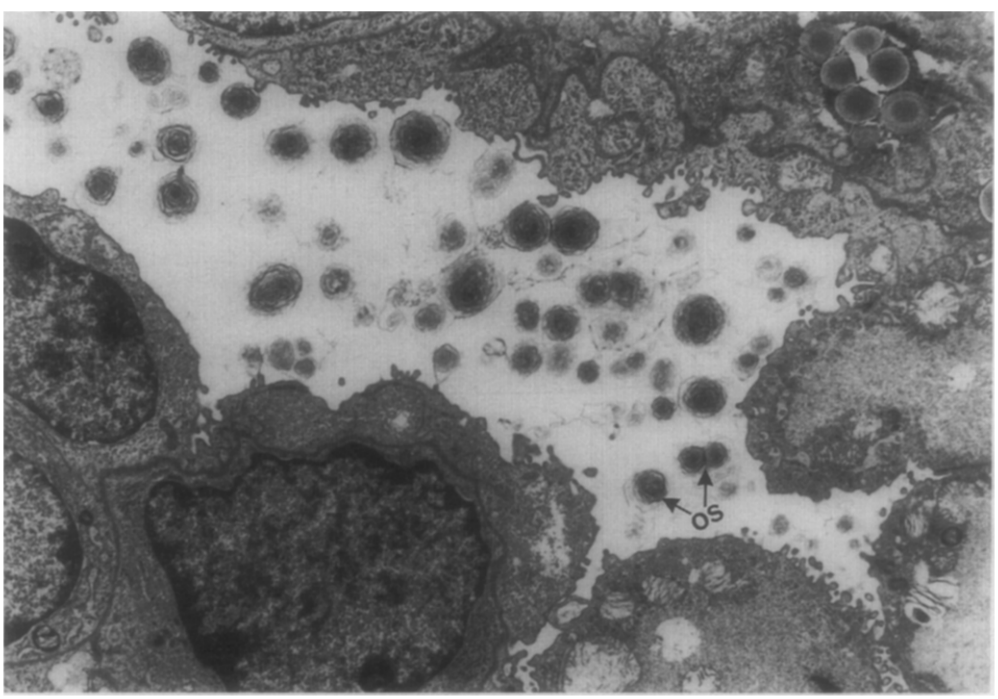

Fig. 1. Low power electron micrograph of dog lung with acute pancreatitis (x4700). Numerous lamellated osmiophilic spheroids (OS) are present in alveolar space.

accumulation of osmiophilic spheroids in alveolar space accompanied by a decrease in the amount of surfactant dipalmitoyl phosphatidylcholine in lung have previously been observed by us in lung damage due to aflatoxin toxicity (9). We proposed earlier a hypothesis that in respiratory distress, there is some association between the accumulation of these osmiophilic spheroid structures and insufficiency of surfactant (9). Such a correlation has also been found in oxygen toxicity (16) and traumatic shock (17). It is possible that in ARDS associated with acute pancreatitis, there is an increase in the catecholamine stimulated secretion of surfactant by the alveolar type II cells, and this hypersecretion causes accumulation of osmiophilic spheriods. Therefore, as a defensive mechanism, certain cell types, possibly macrophages may accumulate and secrete more phospholipase A_2 in the alveoli and thus causing increased breakdown of surfactant phospholipid. Such accumulation of macrophages in the alveoli has been documented in tramatic shock (17). The increase in this activity of phospholipase A_2 causes a reduction in the recovery of useful surfactant and an increase in the level of lysolecithin which may act as an antisurfactant or cellular toxin and be responsible for the development of interstitial alveolar edema and subsequent pulmonary distress. Thus production of acute haemorrhagic pancreatitis in dogs by transduodenal injection of autologous bile into the main pancreatic duct may serve as a model of Adult Respiratory Distress Syndrome.

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