

Pancreatic and synovial type phospholipases A2 in serum samples from patients with severe acute pancreatitis

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Abstract

Phospholipase A2 (PLA2) is the rate limiting enzyme in the formation of prostaglandins and probably plays a key part in the pathology of various inflammatory diseases. In acute pancreatitis, the catalytic activity of PLA2 in serum correlates with the severity of the disease. The cellular source of the catalytically active PLA2 in serum of patients suffering from acute pancreatitis and other diseases is unknown. Immunoassays for the measurement of pancreatic group I PLA2 and non-pancreatic synovial type group II PLA2 have recently been developed and the present study investigated the presence of group I and group II PLA2s in serum samples from 36 patients with severe acute pancreatitis. The catalytic activity of PLA2 showed a highly significant correlation with the concentration of synovial type PLA2 ($r=0.939$, $p=0.001$) but not with the concentration of pancreatic PLA2 ($r=0.067$, $p=0.698$). The results suggest that pancreatic PLA2 circulates mostly as inactive enzyme in patients with acute pancreatitis whereas synovial type PLA2 is responsible for the increased catalytic activity of the enzyme and thus might be associated with the pathophysiology of the disease.

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Phospholipase A2 (PLA2) is a lipolytic enzyme that hydrolyses phospholipids – for example, phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol – into corresponding lysocompounds. PLA2 is the rate limiting enzyme in the early steps of eicosanoid synthesis, and therefore is considered to play a key part in the production of inflammatory mediators. The enzyme is widely distributed in various tissues and cells; in fact, it is said that PLA2 has been found in every tissue studied. Rich sources for the isolation of PLA2 are pancreatic tissue and juice, and snake and bee venoms.^{1,2}

Pancreatic and venom PLA2s have been divided into two groups on the basis of the amino acid sequences of the enzyme proteins.³ Group I PLA2s contain a cysteine at position 11 forming a disulphide bridge with a cysteine at position 77. Group II PLA2s lack this disulphide bridge and the corresponding cysteines. Group I PLA2s are those found in the mammalian pancreas and venoms from *Elapidae* (cobra) and *Hydrophiidae* (sea snakes). Group II PLA2s are found in venoms from *Crotalidae* (rattle snakes) and *Viperidae* (old-world vipers).⁴ The PLA2 found in synovial fluid is a group II PLA2.⁵

It has been postulated that PLA2 is associated with the pathology of various diseases, such as acute pancreatitis, septic shock, and multiple injuries involving infection, tissue damage, or inflammation.^{6–10} In acute pancreatitis, the catalytic activity of PLA2 is increased in serum, especially in the severe necrotising form of the disease.¹¹ The concentration of immunoreactive pancreatic PLA2 in serum is also increased, but this increase only partly explains the abnormal catalytic activity of PLA2 in serum samples from patients with acute pancreatitis.¹² This finding indicates that the catalytically active PLA2 in serum in acute pancreatitis not only originates from the pancreas but also from other sources. The cellular source of the non-pancreatic PLA2 found in serum is unknown as yet.

We have recently developed immunoassays for the measurement of pancreatic group I PLA2¹³ and non-pancreatic synovial type group II PLA2¹⁴ in serum and other body fluids. The purpose of the present study was to investigate the presence of group I and group II PLA2s in serum samples from patients with acute pancreatitis.

Materials and methods

PATIENTS

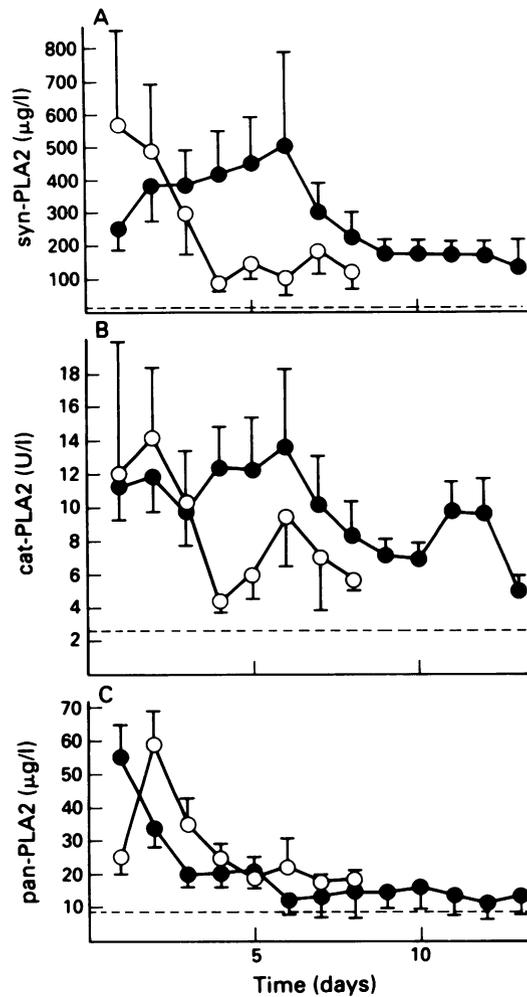
Thirty six consecutive patients treated for severe acute pancreatitis and its complications in the intensive care unit of the University Central Hospital of Turku were included in the study. Inclusion criteria were the classical clinical findings of acute pancreatitis (abdominal pain and tenderness, nausea, and vomiting) and more than a twofold increase in urinary amylase activity (in 34 patients the increase was more than threefold). Contrast enhanced computed tomography ($n=29$), or operation ($n=15$), or both confirmed the diagnosis in 32 patients and it was confirmed by ultrasound scan in the remaining four patients. According to those examinations there were 21 necrotising and 15 oedematous episodes of acute pancreatitis. The mean age of the patients was 42 (range 24–72) years and there were 32 men and four women. The aetiology was alcohol abuse in 26, gall stones in five, and other or unknown in five patients. There were 29 first attacks and seven repeat attacks. The mean duration of stay in the hospital was 19 (range 7–140) days and in the intensive care unit seven (3–20) days. Twenty two patients managed with spontaneous breathing of 28–40% oxygen and the others needed mechanical ventilation. Serum creatinine values were above normal (<120 $\mu\text{mol/l}$ in men and

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Time course of syn-PLA2 (A), cat-PLA2 (B), and pan-PLA2 (C) in patients with oedematous (○) or necrotising (●) episodes of acute pancreatitis (mean (SEM)). The dashed horizontal lines indicate the upper limits of the reference intervals (syn-PLA2 11 µg/l, cat-PLA2 2.5 U/l, and pan-PLA2 9 µg/l).

<100 µmol/l in women) in 10 patients. The mean simplified acute physiology score¹⁵ of the patients was 11, indicating that the mean risk of death was 24.5%. The C-reactive protein value on admission to the intensive care unit was >100 mg/l in all patients except one. Three patients had a positive blood culture (*Staphylococcus aureus*, *Bacillus cereus*, *Bacillus megaterium*, *Aspergillus fumigatus*). Four patients died of acute pancreatitis and its complications.

METHODS

The catalytic activity of amylase in urine was measured with a commercial kit (Merck, Germany), the concentration of C-reactive protein (CRP) in serum by immunoturbidimetry and the concentration of creatinine in serum with a commercial kit (Boehringer Mannheim, Germany). The catalytic activity of PLA2 (cat-PLA2) in serum was assayed with micellar radioactive phosphatidylcholine as substrate.¹⁶ The concentration of pancreatic (group I) PLA2 (pan-PLA2) in serum was measured by a time resolved fluoroimmunoassay with a monoclonal (mouse) antibody raised against human pancreatic PLA2.¹³ The concentration of synovial

type (group II) PLA2 (syn-PLA2) in serum was measured by a time resolved fluoroimmunoassay with a polyclonal (rabbit) antibody raised against recombinant syn-PLA2.¹⁴ Serum samples were obtained routinely daily during the treatment in the intensive care unit. The samples were stored at -20°C until assayed.

STATISTICAL ANALYSIS

Student's *t* test and Pearson's correlation were used for statistical analysis.

Results

The Figure shows the time courses of syn-PLA2, cat-PLA2, and pan-PLA2 in serum samples from patients with oedematous or necrotising episodes of acute pancreatitis. The mean values were considerably increased above the corresponding reference intervals during the first three days in the intensive care unit. The syn-PLA2 and cat-PLA2 values changed in concert, whereas the pan-PLA2 values were increased at the early stages of the disease and then decreased rapidly. The syn-PLA2 and cat-PLA2 values of patients with the necrotising form of acute pancreatitis had a slight tendency to increase during the first six days whereas the values in patients with oedematous acute pancreatitis decreased. The differences in the syn-PLA2, cat-PLA2, or pan-PLA2 values between the groups of patients with oedematous and necrotising acute pancreatitis were not significant.

The cat-PLA2 values had a highly significant correlation with the syn-PLA2 values in the regression analysis ($r=0.939$, $p=0.001$), whereas there was no correlation with pan-PLA2 or CRP or between pan-PLA2 and CRP (Table).

Discussion

In 1961 Zieve and Vogel¹⁷ reported increased lecithinase A (phospholipase A2) activities in serum samples from patients with acute pancreatitis. Later, Schmidt and Creutzfeldt¹⁸ found increased concentrations of lysophosphatidylcholine in necrotic pancreatic tissue in acute pancreatitis. Subsequently, it was postulated that pancreatic PLA2 might play an important part in the pathology of acute pancreatitis.^{6,7,19} Schröder and coworkers²⁰ reported that the increase in the catalytic activity of PLA2 in serum correlated with the severity of acute pancreatitis. Experimental results indicated that the enzymatically active PLA2 found in serum in experimental porcine pancreatitis might

Correlations between syn-PLA2, pan-PLA2, cat-PLA2, and C-reactive protein (CRP) in serum samples from patients with severe acute pancreatitis

| | No of patients | r | p Value* |
|---------------------|----------------|-------|----------|
| syn-PLA2 v cat-PLA2 | 36 | 0.939 | 0.001 |
| syn-PLA2 v pan-PLA2 | 36 | 0.003 | 0.985 |
| syn-PLA2 v CRP | 30 | 0.209 | 0.267 |
| pan-PLA2 v cat-PLA2 | 36 | 0.067 | 0.698 |
| pan-PLA2 v CRP | 30 | 0.009 | 0.961 |
| cat-PLA2 v CRP | 30 | 0.213 | 0.258 |

*Significance of Pearson's correlation.

originate from the pancreas.²¹ Findings from surgically treated human cases did not, however, substantiate the pancreatic origin of the catalytically active PLA2 present in serum in acute pancreatitis.²⁰ When specific immunoassays for the measurement of PLA2 became available,^{12,22} it was found that the concentration of immunoreactive pan-PLA2 is nearly always increased in the early stages of acute pancreatitis.²³ The catalytic PLA2 activity was not invariably removed from serum, however, by immunoadsorption with a specific antipancreatic PLA2 antibody.²⁴ Therefore the source of the circulating PLA2 in acute pancreatitis was not always the pancreas. Also, the severity of the disease did not correlate with the increase in the immunoreactive pan-PLA2 in serum, whereas the increase in the catalytic activity of PLA2 effectively separated the severe necrotising forms from the mild oedematous forms of acute pancreatitis.¹¹ Moreover, the present study indicated that the syn-PLA2 and cat-PLA2 values of patients with a necrotising form of acute pancreatitis had a tendency to remain increased for a longer time than the values in patients with oedematous acute pancreatitis.

We found a highly significant correlation between the catalytic activity of PLA2 and the concentration of syn-PLA2, but not pan-PLA2, in serum from patients with severe acute pancreatitis in the present study. Thus pan-PLA2, which is readily released from the pancreas into blood in acute pancreatitis, circulates mostly as inactive enzyme. The changes in cat-PLA2 and syn-PLA2 values may reflect the clinical state of the patients, because we found recently that the increases in the concentrations of cat-PLA2 and syn-PLA2 were associated with pulmonary and renal complications in acute pancreatitis.²⁵ The present results confirm earlier findings^{11,23} that pan-PLA2 values increase at the early stages of the disease and then rapidly return to normal. The early increase of pan-PLA2 values probably reflects the time of destruction of pancreatic tissue.

Group II PLA2s occur in synovial fluid, platelets, placenta, and inflammatory cells and exudate.^{5,26} Besides acute pancreatitis, increased catalytic activity of PLA2 in serum is associated with many diseases involving infection, tissue destruction, and inflammation including septic shock, rheumatoid arthritis, and multiple injuries.⁸⁻¹⁰ Ourselves¹⁴ and others²⁷ have recently found, by new immunoassays, increased concentrations of syn-PLA2 in serum samples from patients with septic fever and infections. The blood culture was positive in three patients in the present study. In these patients the concentration of syn-PLA2 in serum was very high (>1000 µg/l) at the time of the positive cultures.

The cellular source of group II PLA2 found in serum samples from patients with acute pancreatitis and other inflammatory diseases remains unknown. It was proposed recently^{28,29} that group II PLA2 might represent an acute phase reactant. Hepatoma cells in culture secrete PLA2 into the culture medium when stimulated by interleukin 6.²⁸ We have recently found by our immunoassay considerable amounts of syn-PLA2 in synovial fluid and seminal plasma, and

detected the enzyme by immunohistochemistry in cartilage and Paneth cells of the intestinal mucosa (Nevalainen T J and Haapanen T J. Unpublished data).

The determination of immunoreactive synovial-type PLA2 as described in this paper might be helpful in the early assessment of the severity of acute pancreatitis. The therapeutic implications of the present findings are to be established in acute pancreatitis. PLA2 has been considered earlier to act mainly as a harmful agent in the pathology of various inflammatory diseases including acute pancreatitis.⁶⁻¹⁰ Pancreatic PLA2 seems to be non-toxic to pancreatic acinar cells, however,^{30,31} although toxic effects have also been found.³² Human group II PLA2 purified from cartilage seems non-toxic to cells in culture (Nevalainen T J and coworkers. Unpublished results). As an acute phase reactant, the role of group II PLA2 in infections and inflammatory diseases might be related to the host's defence against invading micro-organisms and tissue destruction.

We conclude that the catalytic activity of PLA2 in serum in acute pancreatitis is due to the presence of a synovial type group II PLA2. The PLA2 activity is not dependent on the concentration of pancreatic group I PLA2 in serum.

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