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Group I and Group II Phospholipases A_2 in Serum in Uraemia

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Summary: Time-resolved fluoroimmunoassays were used for the detection of pancreatic group I and synovial-type group II phospholipases A_2 in sera of patients suffering from chronic renal failure before and after haemodialysis. The concentration of group I phospholipase A_2 was ten-fold higher in sera of uraemic patients than in healthy controls. There was no significant difference in the concentrations of group I phospholipase A_2 in serum before and after haemodialysis. The concentration of group II phospholipase A_2 was only marginally increased in sera of uraemic patients, compared with healthy controls. There was no significant difference in the concentrations of group II phospholipase A_2 before and after haemodialysis. The results indicate that the metabolism of group I phospholipase A_2 differs from that of group II phospholipase A_2 in chronic renal failure.

Introduction

Extracellular phospholipases A_2[1]) have been classified into two types:

pancreatic group I phospholipase A_2 and

synovial-type group II phospholipase A_2

on the basis of the primary structure of the enzyme protein. Group I phospholipases A_2 contain a cysteine at position 11 that forms a disulphide bridge with a cysteine at position 77, whereas group II phospholipases A_2 lack these cysteines and the corresponding disulphide bridge (1). Group I phospholipase A_2 is secreted by pancreatic acinar cells as an enzymatically inactive proenzyme into the duodenum, where it is activated by trypsin (2,3). Increased concentrations of immunoreactive group I phospholipase A_2 have been found in sera of patients suffering from acute pancreatitis (4, 5) and pancreatic cancer (4). Increased phospholipase A_2 catalytic activities and concentrations of group II phospholipase A_2 have been measured in serum in sepsis (6, 7) and in synovial fluid and serum in rheumatoid arthritis and osteoarthritis (8). The source of the circulating group II phospholipase A_2 is unknown.

High activities and concentrations of pancreatic enzymes have been measured in peripheral blood samples of patients suffering from chronic renal failure (9, 10). The catalytic activity of phospholipase A_2 is more than eight-fold above normal in uraemic plasma (11). The concentration of group I phospholipase A_2 in serum is elevated in patients suffering from acute renal dysfunction (12) and chronic renal failure (13).

The purpose of the present study was to investigate the concentrations of pancreatic and non-pancreatic phospholipases A_2 by specific immunoassays for group I phospholipase A_2 (14) and group II phospholipase A_2 (7) in sera of patients suffering from chronic renal failure. Measurements were done on serum samples taken before and after haemodialysis and compared with values of healthy individuals.

[1]) Enzyme:
Phosphatide 2-acylhydrolase, phospholipase A_2 (EC 3.1.1.4)

Materials and Methods

Serum samples

Blood samples were taken immediately before and after haemodialysis from 10 patients (6 men and 4 women, average age 50 years; range 24–72 years) suffering from chronic renal failure (five patients had chronic nephritis, one had *Wegner*'s granulomatosis, gout or malignant hypertension and two patients had chronic renal failure of unknown etiology). There were no clinical signs of pancreatic disease in these patients. Serum was separated and stored at −20 °C until assayed. Reference material was collected from 20 healthy blood donors (6 men and 14 women, average age 37 years; range 33–40 years) for the group I phospholipase A_2 assay and from 27 blood donors (21 men and 6 women, average age 31 years; range 24–66 years) for the group II phospholipase A_2 assay, and treated identically to patient sera.

Apparatus

Microtitre plates were washed in a 12-well aspirating-washing device (Wellwash 4, Denley, Billingham, England) and incubated in a shaking device (Wellmix 3). Fluoroscence was measured with an Arcus fluorometer (Wallac, Turku, Finland) equipped with an external computer. Data were handled with a MultiCalc data management software (Wallac).

Standards

Human pancreatic group I phospholipase A_2 and recombinant synovial-type group II phospholipase A_2 stock solutions were diluted with a time-resolved fluoroimmunoassay (TR-FIA) assay buffer (Wallac) to give five standard concentrations (0.25, 1.5, 9.0, 54 and 324 μg/l). Group I phospholipase A_2 was purified from human pancreas as described elsewhere (15). Recombinant synovial-type group II phospholipase A_2 was prepared as described elsewhere (16).

Time-resolved fluoroimmunoassay

Time-resolved fluoroimmunoassays for group I phospholipase A_2 and group II phospholipase A_2 were performed as described earlier (7, 14, respectively).

Statistical analysis

Student's t-test was used unless the difference of variances of variables warranted the use of *Mann-Whitney*'s U-test. Tests were performed by using the BMDP statistical software package.

Results

Group I phospholipase A_2

Patients suffering from chronic renal failure had tenfold increased group I phospholipase A_2 values compared with the reference group, both before and after haemodialysis (fig. 1). The mean concentration of group I phospholipase A_2 in the reference group was 4.0 μg/l (range from 1.7 μg/l to 5.6 μg/l, n = 20). The mean concentration of group I phospholipase A_2 before haemodialysis was 38.1 μg/l (range from 11.1 μg/l to 69.2 μg/l, n = 10) and after haemodialysis 40.4 μg/l (range from 12.9 μg/l to 95.1 μg/l) in patients with chronic renal failure. The difference between the reference samples and the samples taken before and after haemodialysis was statistically significant ($p < 0.00004$). The difference between the samples taken before and after haemodialysis was not statistically significant ($p = 0.832$).

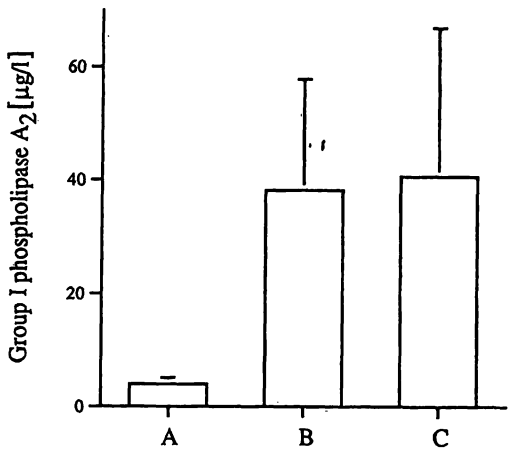

Fig. 1. Concentrations of group I phospholipase A_2 (mean, SD) in the sera of 20 healthy individuals (A, 4.0 ± 1.1 μg/l) and 10 patients suffering from chronic renal failure before (B, 38.1 ± 19.5 μg/l) and after (C, 40.4 ± 26.3 μg/l) haemodialysis.

Group II phospholipase A_2

The mean concentration of group II phospholipase A_2 in the reference group was 5.0 μg/l (range from 2.1 μg/l to 25.3 μg/l, n = 27). The mean concentration of group II phospholipase A_2 before haemodialysis was 7.6 μg/l (range from 3.3 μg/l to 20.4 μg/l, n = 10) and after haemodialysis 8.8 μg/l (range from 3.6 μg/l to 25.5 μg/l) in patients with chronic renal failure. There was no significant difference between group II phospholipase A_2 values in patients with chronic renal failure before haemodialysis and in the reference group ($p = 0.137$, fig. 2). The difference between the concentrations of group II ATP phospholipase A_2 in samples taken after haemodialysis and those of the reference group was marginally significant ($p = 0.046$). The difference between the samples taken before and after haemodialysis was not significant ($p = 0.641$).

Discussion

The role of phospholipase A_2 in acute pancreatitis has been discussed extensively (4, 17, 18). Recent findings indicate that non-pancreatic (synovial-type) group II phospholipase A_2 is responsible for the increased catalytic activity of phospholipase A_2 in serum in acute pancreatitis (19, 20), as in other inflammatory diseases (6, 7, 21).

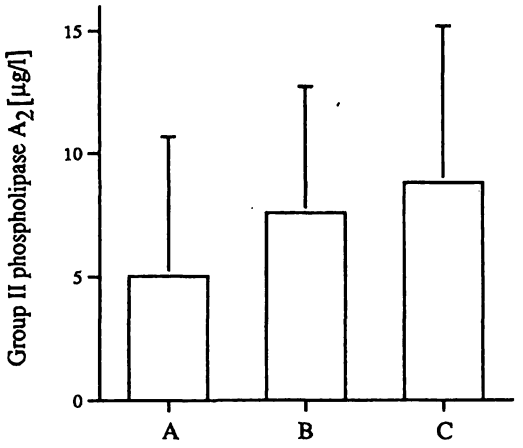

Fig. 2. Concentrations of group II phospholipase A_2 (mean, SD) in the sera of 27 healthy individuals (A, 5.0 ± 4.3 µg/l) and 10 patients suffering from chronic renal failure before (B, 7.6 ± 5.1 µg/l) and after (C, 8.8 ± 6.3 µg/l) haemodialysis.

Patients suffering from chronic renal failure had increased catalytic activities of phospholipase A_2 in plasma (11) and increased concentrations of group I phospholipase A_2 in serum as determined by a radioimmunoassay (13). Our results confirm these findings. There was a statistically significant, ten-fold increase in the concentrations of group I phospholipase A_2 in sera of patients with chronic renal failure compared with reference material from healthy individuals. The treatment of uraemia by haemodialysis did not change the concentrations of group I phospholipase A_2 in the sera of the present patients. Our findings also confirm that the activities of pancreatic enzymes in serum increase in patients with end-stage renal disease (10) and chronic renal failure without pancreatic disease (9). There were no changes in the elevated amylase activities during haemodialysis in these patients. Our patients had advanced chronic renal failure, but no signs of pancreatic disease. Experimental observations on nephrectomized and intact rats indicate that group I phospholipase A_2 injected intravenously is eliminated from the circulation mainly by the kidneys (22).

The elevated catalytic activity of phospholipase A_2 in serum is caused by an increase in the concentration of group II phospholipase A_2 in various inflammatory diseases (6, 7, 19–21). The concentration of group II phospholipase A_2 in sera of the present patients with chronic renal failure was similar to that in the samples of healthy individuals. Haemodialysis did not change the concentration of group II phospholipase A_2. These findings suggest that group I and group II phospholipases A_2 are metabolized differently when their renal elimination from blood plasma is disturbed.

It can be concluded that the concentration of group I phospholipase A_2 is markedly increased in serum in chronic renal failure, whereas the concentration of group II phospholipase A_2 remains within the reference interval. Haemodialysis does not affect the concentrations of group I and group II phospholipases A_2 in serum in patients with chronic renal failure.

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