Immunoreactive Pancreatic Phospholipase A2 and Catalytically Active Phospholipases A2 in Serum from Patients with Acute Pancreatitis

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Measuring the content of immunoreactive pancreatic phospholipase A2 (PLA2; EC 3.1.1.4) and the catalytic activity of PLA2 in serum samples from five patients with acute pancreatitis, we found no correlation between these two measurements overall. To test the specificity of the method for catalytic PLA2, we measured PLA2 activity in serum samples before and after immunoadsorption with an antiserum to human pancreatic PLA2. The results suggest the presence of at least two immunologically distinct PLA2 enzyme proteins in sera from these patients. One of the enzymes is pancreatic in origin and may exist in active, inactive, or inhibited form. The activity profile of the second PLA2 enzyme in serum during acute pancreatitis differs from that for other common pancreatic enzymes. In the present experiment, the catalytic activity was not removed by treatment with the anti-human pancreatic PLA2 antiserum. The source of this second PLA2 activity is unknown. Some samples contained increased activities of both PLA2 forms.

Additional Keyphrases: immunoadsorption • pancreatic disease • time-resolved fluoroimmunoassay • isoenzymes • enzyme activity

Several reports suggest a role for phospholipase A2 (PLA2; EC 3.1.1.4) in the pathogenesis of acute pancreatitis (1, 2). Both the concentration of immunoreactive phospholipase A2 (IR-PLA2) and the catalytic PLA2 activity (CA-PLA2) in serum increase in clinical (3-7) and experimental pancreatitis (8, 9). Positive correlations have been reported between the concentrations in serum of both IR-PLA2 and CA-PLA2 and the severity of the disease (3, 5, 6, 10, 11), despite the fundamental differences between these two ways to detect PLA2. The immunoassay measures, specifically and quantitatively, the secretory PLA2 originating in pancreas (4, 12). The enzyme protein measured, however, may be in the form of a proenzyme, and may be catalytically active or inactive. Conversely, the specificity of the enzymological measurement of pancreatic PLA2 is poor: different kinds of PLA2 activity in serum will be quantified. However, this method will not measure proenzyme, inactive enzyme, or inhibited enzyme.

In this study, we measured IR-PLA2 and CA-PLA2 in sera of patients with clinically documented acute pancreatitis. We also assayed all IR-PLA2 from the serum samples with an antiserum to human PLA2 and measured the CA-PLA2 activity before and after the immunoadsorption, to study the specificity of the enzymatic measurement. Catalytically active PLA2's other than those originating in pancreas appear to be present in sera of patients with acute pancreatitis.

Materials and Methods

Methods. Immunoreactive PLA2 was measured by a solid-phase time-resolved fluoroimmunoassay described earlier (4). The reference interval (mean ± SD) for IR-PLA2 in serum was 1.8 to 9.2 µg/L, as determined earlier (5). The catalytic activity of PLA2 was measured by the method of Shakir (13) as modified by Schädlisch et al. (14). PLA2 activity is expressed in U/L, 1 unit (U) being defined as that activity liberating 1 µmol of fatty acid per minute. PLA2 was immunoadsorbed from serum samples by use of anti-human PLA2 antiserum coated onto microtiter wells, prepared as described earlier (4). A 100-µL aliquot of serum was incubated in a well for 1 h, then transferred to a fresh well. This immunoadsorption process was repeated until the serum sample was free of IR-PLA2, as determined by time-resolved fluoroimmunoassay.

Materials. Silicic acid and sodium deoxycholate were purchased from Merck AG, Darmstadt, F.R.G.; L-α-dipalmitoyl phosphatidylcholine and porcine phospholipase A2 from Sigma Chemical Co., St. Louis, MO 63178. L-α-Dipalmitoyl-

[2-palmitoyl-1-14C]Phosphatidylcholine was obtained from NEN, Dreieich, F.R.G. Human pancreatic PLA2 was purified from cadaver pancreas, and antisera to human pancreatic PLA2 was raised in rabbits (15).

Subjects. Five patients (four men and one woman) with clinically diagnosed acute pancreatitis were studied (Table 1). All patients recovered. Three who underwent pancreatic resection were found to have hemorrhagic pancreatitis.

Results

The normal range for CA-PLA2 was determined by analyzing sera from 15 healthy persons. The mean value was 0.892 U/L. The reference interval (mean ± 2 SD) for CA-PLA2 in this population was 0.296–1.489 U/L.

Figure 1 shows the changes in IR-PLA2 and CA-PLA2 values in serum samples from patients with acute pancreatitis, after hospital admission. The serum IR-PLA2 was above normal in the first sample after admission in all cases, but the serum CA-PLA2 was not increased in two cases (patients 1 and 2). The activity profile of IR-PLA2 differs from that of CA-PLA2 in the serum samples of patients 4 and 5.

There was no correlation between the serum IR-PLA2 and CA-PLA2 overall in this small set of samples (Figure 2). However, in the serum samples from patients 2 and 3, there was a correlation between IR-PLA2 and CA-PLA2: CA-PLA2 = 0.026 IR-PLA2 + 0.699 U/L (n = 7, r = 0.96).

The values for IR-PLA2 and CA-PLA2 in the serum of all patients before and after immunoadsorption with the anti-human PLA2 antiserum are plotted as a function of days after hospital admission in Figure 3. The PLA2 adsorbed from the serum of patient 1 was almost totally enzymatically inactive. The PLA2 in the sera of patients 2 and 3 was
active and almost completely adsorbed with the antiserum. We saw a good correlation between the IR-PLA₂ and CA-
PLA₂ in the serum samples from these two patients (Figure 2). PLA₂ in the serum of patient 4 on the day of admission was active and most of it was adsorbed onto the antibody. In the samples collected on the following days, IR-PLA₂ was adsorbed onto the antibody but the catalytic activity did not disappear. Also, in the serum of patient 5 the main part of the catalytic activity was not adsorbed by the antiserum. The catalytic activity in this case was much more increased than was the immunoreactivity.

Discussion

Our findings indicate that at least two different phospholipases A₂ can be present in the sera of patients with acute pancreatitis. In the sera of the patients 4 and 5, the antiserum against pancreatic PLA₂ does not recognize all active phospholipase A₂. One form of the enzyme in the sera of the patients increased or stayed increased while the antiserum-specific form gradually decreased as a function of the number of days after hospital admission. Schröder et al. (10) have also reported a similar finding in patients who underwent pancreatic resections. PLA₂ activity remained high or even increased further during the post-operative period, although serum amylase activity and other laboratory values returned to normal. Usually the pancreatic enzymes (amylase, lipase, trypsin) liberated into the serum of patients with acute pancreatitis are normalized gradually and in a similar manner. The PLA₂ activity remaining after immunoabsorption behaves differently. This suggests that the enzyme responsible for it might not be pancreatic in origin. The coexistence of two PLA₂'s partly explains the lack of the correlation between IR-PLA₂ and CA-PLA₂. On the other hand, the IR-PLA₂ in the serum of one patient was catalytically inactive, although it was much above normal. The enzyme may be denatured, or present as a proenzyme. Also, the presence of inhibitors or other factors in serum could interfere with the CA-PLA₂ measurement. Recently, Matsuda et al. (6) reported the increase in IR-PLA₂ in the serum of patients with severe acute pancreatitis to be greater than would be calculated from the catalytic activity. Activation of PLA₂ with trypsin did not increase the enzymatic activity to the level expected.

Table 1. Patients

<table>
<thead>
<tr>
<th>Pat. no.</th>
<th>Age, y</th>
<th>Attack no.</th>
<th>Etiology</th>
<th>History</th>
<th>Operation</th>
<th>Operative finding</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>4</td>
<td>Unknown</td>
<td>Prisoner, no access to alcohol</td>
<td>Not operated</td>
<td></td>
<td>Uneventful recovery</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>1</td>
<td>Alcoholic</td>
<td>Chronic alcoholic</td>
<td></td>
<td>Pancreatic resection</td>
<td>Post-operative biliary peritonitis starting on day 16 Recovered</td>
</tr>
<tr>
<td>3</td>
<td>35</td>
<td>3</td>
<td>Alcoholic</td>
<td>Chronic alcoholic</td>
<td></td>
<td>Pancreas hemorrhagic and necrotic</td>
<td>Septic fever starting on day 17 Recovered</td>
</tr>
<tr>
<td>4</td>
<td>56</td>
<td>1</td>
<td>Biliary</td>
<td>Gallstones</td>
<td>Pancreatic resection 80%, day 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>2</td>
<td>Alcoholic</td>
<td>Chronic alcoholic</td>
<td></td>
<td>Pancreas hemorrhagic and necrotic</td>
<td></td>
</tr>
</tbody>
</table>

*All men, except patient 4.

Fig. 1. Changes during hospitalization in the concentration of IR-PLA₂ (□) and catalytic activity of PLA₂ (△) in sera from patients with acute pancreatitis

Ordinate: multiples of increase beyond the upper limit of normal range

Fig. 2. Correlation of serum PLA₂ activity with the PLA₂ immunoreactivity in patients 1–5

The regression line presented is for samples from patients 2 and 3
Our results indicate the presence of two different PLA₂ enzymes in the serum of patients with acute pancreatitis. The co-existence of two PLA₂'s with different profiles for their increases should be taken into consideration in the interpretation of the results of methods measuring the catalytic activity of PLA₂ in serum of patients with acute pancreatitis. The source of the nonpancreatic PLA₂ activity is unknown.

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References