Immunoreactive Phospholipase $A_2$ in Serum in Acute Pancreatitis and Pancreatic Cancer

Timo J. Nevalainen,¹ Jarkko U. Eskola,²,6 Allan J. Aho,³ V. Tapani Havila,³ Timo N.-E. Lövgren,⁴ and Velko Näntö³

Immunoreactive phospholipase $A_2$ (EC 3.1.1.4) was measured by a new sensitive time-resolved fluoroimmunoassay in the serum of 58 healthy subjects and 103 patients with acute pancreatitis. Patients with acute pancreatitis were grouped according to the etiology and clinical severity of the disease. The mean phospholipase $A_2$ concentration in the reference (healthy) group was 5.5 (SD 1.9) μg/L. In acute pancreatitis the mean phospholipase $A_2$ concentration was increased on the first day after hospital admission in all groups, and returned to normal somewhat more slowly than did serum amylase, especially in the patients with severe alcoholic pancreatitis. In this latter group the mean concentration of serum phospholipase $A_2$ on the first day was 42.6 (SD 29.5) μg/L. In patients with pancreatic cancer, serum phospholipase $A_2$ was 29.2 (SD 21.3) μg/L. The phospholipase $A_2$ and amylase values were closely associated in all groups. The clinical sensitivities were 90.9% for severe alcoholic pancreatitis and 87.5% for pancreatic cancer. Immunochemical determination of phospholipase $A_2$ in serum provides fast and specific detection of injury to pancreatic acinar cells. In addition to the early diagnosis of acute pancreatitis, follow-up determinations of phospholipase $A_2$ seem to be useful in differentiating between mild and severe forms of pancreatitis.

Additional Keyphrases: pancreatic disease · pancreatic enzymes · reference interval · time-resolved fluoroimmunoassay · cancer · amylase

Several studies suggest that the activation of pancreatic phospholipase $A_2$ (PLA$_2$; phosphatidylcholine 2-acylhydro- lase, EC 3.1.1.4) and its release from injured acinar cells play an important role in the pathogenesis of acute pancreatitis (1). This enzyme hydrolyzes phospholipids—e.g., the lecithin in cellular membranes, pulmonary surfactant, and bile. We described recently the purification and some biochemical and immunological properties of human pancreatic PLA$_2$ (2), and developed a time-resolved fluoroimmunoassay for its determination in human serum (3). In the present study we investigated the clinical utility of a laboratory test for serum PLA$_2$ by determining its concentration in serum of healthy persons and in patients with acute pancreatitis and pancreatic cancer, and comparing it with the activity of amylase in serum.

Materials and Methods

Materials

PLA$_2$ purified from human cadaver pancreas as described previously (2), was homogeneous as judged from sodium dodecyl sulfate/polyacrylamide gel electrophoresis and "high performance" ion-exchange chromatography. Antisera to PLA$_2$ were raised in rabbits by immunizing them with this enzyme preparation. We used a one-incubation, multisite, solid-phase fluoroimmunoassay as described earlier (3) to determine the quantities of immunoreactive PLA$_2$ in serum. To measure fluorescence, we used a single-photon-counting time-resolved fluorometer (1230 Arcus; LKB Wallac, Turku, Finland) (4). Amylase activity was determined by a photometric method with Blue Starch polymer (Phadebas®; Pharmacia Fine Chemicals, Uppsala, Sweden) substrate, according to the manufacturer's instructions.

Subjects

Our study population consisted of patients treated for pancreatic disease during the period from September 1982 to August 1984 in the Department of Surgery, Turku University Central Hospital (Table 1). Six patients had two or three separate episodes of acute pancreatitis during the study. Serum was collected from the patients for amylase determinations during hospitalization, and stored at $-20^\circ$C for later measurement of PLA$_2$. The diagnosis of carcinoma was verified by biopsies at laparotomy. The serum samples had been obtained from these patients before the operation. PLA$_2$ concentrations and amylase activities in serum were stable at $-20^\circ$C for at least six months.

The diagnosis of acute pancreatitis was based on typical clinical findings (sudden abdominal pain, tenderness in the epigastrium, fever) and above-normal amylase activity in serum, urine, or both. Only patients with a known alcoholic or biliary etiology were included in the study. They were further divided into mild and severe pancreatitis according to the course and outcome of the disease (see Table 1). Values for amylase (serum or urine) were not considered in assessing severity.

The control group consisted of 58 normal, ostensibly healthy volunteers (Table 1).

We used the following criteria in classifying the clinical severity of the pancreatitis:

Mild pancreatitis: Symptoms and signs of acute pancreatitis disappeared within four days after the admission to the
hospital. Treatment with intravenous fluid was given during this period and peroral feeding was started gradually by the third to fourth day.

Severe pancreatitis: Symptoms and signs such as abdominal distention, ascites, and the development of some complication persisted for five days or more after admission to the hospital, and the patients were treated with intravenous fluids and analgesics for more than four days.

Statistical Analysis

We analyzed the differences between the groups by non-parametric tests. The overall significance of differences was calculated with the Kruskal-Wallis test; if the differences were significant ($p < 0.05$), we also tested the differences between the groups pairwise with the Mann-Whitney U test. The dependency of PLA$_2$ on age in the reference group was studied by linear regression analysis. The relationship between concentrations of PLA$_2$ amylase was estimated with one-way analysis of covariance, with patients used as the grouping variable (5).

Results

Healthy controls. The concentration of PLA$_2$ in serum was 5.0 (SD 1.9) $\mu$g/L for men, 5.9 (SD 1.7) $\mu$g/L for women, and 5.5 (SD 1.9) $\mu$g/L for the total group (Table 1, Figure 1). The values were not affected by age or sex. The reference interval (mean $\pm 2$ SD) for serum PLA$_2$ in this population was 1.8 to 9.2 $\mu$g/L. In our laboratory the reference interval for serum amylase in healthy subjects is 84 to 309 U/L.

Patients with acute pancreatitis. Serum PLA$_2$ was increased in acute pancreatitis (Figures 2 and 3, Table 2). On the first day after hospital admission the concentration of PLA$_2$ exceeded 3.2 $\mu$g/L in 10 of 11 cases of severe alcoholic pancreatitis, a clinical sensitivity of 90.9%. The sensitivities of PLA$_2$ and serum amylase determinations for detecting different forms of pancreatitis are given in Table 3.

Both PLA$_2$ and amylase showed the highest increase on the first day after hospital admission in all groups with acute pancreatitis. The PLA$_2$ values remained above normal for four to 10 days during hospitalization (Figures 2 and 3, Table 2), markedly longer than the corresponding increase in amylase values. Concentrations of PLA$_2$ were greater in severe alcoholic pancreatitis than in mild alcoholic pancreatitis on days 1, 2, and 3, whereas the corresponding amylase values differed on day 1 only (Figure 2). The differences in serum PLA$_2$ or amylase values between severe biliary pancreatitis and mild biliary pancreatitis were statistically not significant. However, there were statistically significant differences in PLA$_2$ values between patients with severe alcoholic pancreatitis and those with mild biliary pancreatitis ($p < 0.023$) on the second day, and between severe biliary pancreatitis and mild alcoholic pancreatitis ($p < 0.017$), and in amylase values between the latter two groups ($p < 0.012$) on the first day after hospital admission.

Fig. 1. Serum immunoreactive phospholipase A$_2$ content (S-IR-PLA$_2$) in 58 healthy controls
Mean, 5.5 (SD 1.8) $\mu$g/L; range (2 SD), 1.8 to 9.2 $\mu$g/L.

The serum PLA$_2$ and amylase values were closely associated in all pancreatitis groups (Table 4).

Patients with pancreatic carcinoma. In pancreatic carcinoma PLA$_2$ was markedly increased (mean 29.2, SD 21.3 $\mu$g/L, $p < 0.00001$ as compared with the control group). The corresponding serum amylase values were 787 (SD 636) U/L. Values for both PLA$_2$ and amylase in serum were increased in seven of the eight cancer patients (Table 3), but PLA$_2$ was markedly more increased than amylase.

Discussion

Although the diagnosis of acute pancreatitis is based mainly on clinical findings, laboratory tests are of critical value in supporting the diagnosis. However, no simple and reliable laboratory test is available at present that specifically demonstrates damage to pancreatic tissue in acute pancreatitis or other pancreatic diseases. According to our present study, the serum immunoreactive PLA$_2$ is increased at the early stages of acute pancreatitis. Unlike amylase, which is present in serum as pancreatic and salivary isoenzymes, PLA$_2$ most probably is an organ-specific pancreatic enzyme. It has been localized in acinar cells by immunohistochemistry (7), and it disappears from serum after total pancreatectomy (8). Thus PLA$_2$ is potentially an ideal marker of acinar injury.

There is a fundamental difference between measurements of the immunoreactive PLA$_2$ and of the enzymic activity of amylase in serum. The measurement of PLA$_2$ includes the concentrations of active and inactive forms of the enzyme,
Fig. 2. Changes in the mean concentration of PLA₂ (A) and amylase activity (B) in serum in patients with severe alcoholic pancreatitis (SAP) (—) or mild alcoholic pancreatitis (MAP) (——) during hospitalization. The hatched area represents the reference interval. The numbers at the tops of the Figures indicate the no. of patients on the corresponding day in each group. The differences in the PLA₂ values between SAP and MAP groups are statistically significant on days 1, 2, and 3 (p < 0.0024, < 0.013, and < 0.017, respectively); serum amylase values are significantly different between groups on day 1 (p < 0.019).

Fig. 3. Changes in the mean concentration of PLA₂ (A) and amylase or activity (B) in serum in patients with severe biliary pancreatitis (SBP) (—) or mild biliary pancreatitis (MBP) (——) during hospitalization. The differences in the serum amylase or PLA₂ values between SBP and MBP groups are not statistically significant. Reference intervals and no. of patients designated as in Fig. 2.

Table 2. Statistical Comparison of PLA₂ Values (in μg/L) for the Control Group and the Different Patient Groups during Hospitalization

<table>
<thead>
<tr>
<th>Days after admission</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP* n</td>
<td>11</td>
<td>13</td>
<td>10</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>1</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pc(%)</td>
<td>—</td>
<td>—</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.05</td>
</tr>
<tr>
<td>MAP n</td>
<td>10</td>
<td>34</td>
<td>35</td>
<td>28</td>
<td>14</td>
<td>6</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pc(%)</td>
<td>0.01</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.05</td>
<td>0.05</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>SBP n</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pc(%)</td>
<td>0.05</td>
<td>N.S.</td>
<td>0.001</td>
<td>0.01</td>
<td>N.S.</td>
<td>0.01</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MBP n</td>
<td>10</td>
<td>6</td>
<td>9</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>0</td>
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<td></td>
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<tr>
<td>pc(%)</td>
<td>N.S.</td>
<td>0.01</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
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<td></td>
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<tr>
<td>PC n</td>
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</tr>
<tr>
<td>pc(%)</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

*Abbreviations as in Table 1. n No. of patients on each day. pc(%) Significance by Mann-Whitney U test. N.S., not significant.

whereas determination of amylase activity includes only the active forms, and can be affected by several undesirable factors (6).

Several authors have used immunochemical determinations of pancreatic enzymes in serum to diagnose acute pancreatitis. Radioimmunoassays have been developed for serum elastase (9, 10), trypsin (11), chymotrypsin (12), ribonuclease (13), amylase (14), and PLA₂ (8). The determination of immunoactive trypsin in serum has been recommended as an alternative or supplement to the measurement of pancreatic isoamylase (15). Although elastase 1 remains increased for several days after the onset of acute pancreatitis, even after serum amylase activity returns to normal (10), determination of serum elastase content does not help differentiate between acute hemorrhagic and edematous pancreatitis (16).
Table 3. Clinical Sensitivity of Measurements of Serum PLA₂ (µg/L) and Serum Amylase for Diagnosis of Acute Pancreatitis and Pancreatic Cancer on the First Day after Admission

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>No. with PLA₂ &gt;9.2</th>
<th>PLA₂ sensitivity, %</th>
<th>Amylase concn, UL</th>
<th>Amylase sensitivity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP</td>
<td>11</td>
<td>&gt;9.2</td>
<td>90.9</td>
<td>&gt;300</td>
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<tr>
<td>MAP</td>
<td>34</td>
<td>&lt;9.2</td>
<td>58.8</td>
<td>&lt;300</td>
</tr>
<tr>
<td>SBP</td>
<td>3</td>
<td></td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>MBP</td>
<td>10</td>
<td></td>
<td>80.0</td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>8</td>
<td></td>
<td>87.5</td>
<td></td>
</tr>
<tr>
<td>All together</td>
<td>66</td>
<td></td>
<td>72.7</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.

Table 4. PLA₂ As a Function of Amylase Values in Patients with Acute Pancreatitis

<table>
<thead>
<tr>
<th>Degrees of freedom</th>
<th>Regression coefficient</th>
<th>t</th>
<th>p</th>
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<tr>
<td>SAP</td>
<td>54</td>
<td>0.0055</td>
<td>3.14</td>
</tr>
<tr>
<td>MAP</td>
<td>81</td>
<td>0.012</td>
<td>5.11</td>
</tr>
<tr>
<td>SBP</td>
<td>11</td>
<td>0.0075</td>
<td>2.90</td>
</tr>
<tr>
<td>MBP</td>
<td>19</td>
<td>0.0094</td>
<td>6.95</td>
</tr>
<tr>
<td>All groups together</td>
<td>171</td>
<td>0.0077</td>
<td>8.19</td>
</tr>
</tbody>
</table>

Statistical calculations were made with one-way analysis of covariance, with patients as the grouping variable. Abbreviations as in Table 1.

In the present study, the diagnosis of pancreatitis was confirmed by surgery in only a few cases, so we can draw no conclusions as to the value of the PLA₂ determinations in the differential diagnosis of hemorrhagic acute pancreatitis. Increased concentrations of immunoreactive PLA₂ in acute pancreatitis were reported earlier by Eskola et al. (3) and Niisijäms et al. (8). Increased enzymic activities of PLA₂ have been reported in acute pancreatitis (17, 18), but the immunoreactive PLA₂ values in serum cannot be directly correlated with the enzymic activity measurements because enzymically inactive forms (prophospholipase A₂ and possibly degradation products of the enzyme) might also be immunoreactive.

The time-resolved fluoroimmunoassay we used to measure serum immunoreactive PLA₂ (3) is simple and more sensitive than radioimmunoassay and obviates the use of radioactive materials. The mean (±SD) PLA₂ value measured in the control group (5.5 ± 1.9 µg/L, n = 58) corresponds well to the values for PLA₂ measured by radioimmunoassay (5.1 ± 1.7 µg/L, n = 15) (8).

To study the relationship between the etiology and severity of pancreatitis and the increase in serum enzymes during the course of the disease, we divided the patients into groups with mild or severe pancreatitis caused by either alcohol or biliary disorders. The diagnostic sensitivity of serum PLA₂ was excellent in the severe cases of the disease. Its concentration in serum returned normal more slowly than did amylase activity, and was significantly higher in severe alcoholic pancreatitis than in mild alcoholic pancreatitis on days 1–3 (Mann–Whitney test); amylase was significantly higher in severe alcoholic pancreatitis on day 1 only. We found no statistically significant differences between severe and mild forms of biliary pancreatitis with either of the enzymes. By Student's t-test after logarithmic transformation of the data, PLA₂ was significantly higher in the severe than in the mild forms of pancreatitis on several days during the course of the disease; we found no such differences in amylase activities.

Serum concentrations of PLA₂ were markedly increased in the small group of patients suffering from pancreatic carcinoma. This result is most probably due to destruction of pancreatic tissue by the tumor. Thus, determination of PLA₂ might help in the diagnosis of pancreatic cancer, especially when a persistently high concentration is found. However, patients suffering from renal failure also show persistently increased serum PLA₂ values (8), as we also have confirmed (unpublished results).

Our present study shows that PLA₂ concentration in serum is significantly increased at the early stages of acute pancreatitis and remains increased somewhat longer than does serum amylase activity. The increase of serum PLA₂, which may be considered as an organ-specific pancreatic marker, most probably reflects the extent of tissue destruction in the gland, and seems to correlate better with the severity of the disease than does serum amylase activity. Currently, the incubation time in our PLA₂ assay is 1.5 h; for emergency diagnostic purposes, a faster method to measure PLA₂ concentration in serum is needed. A faster screening method is now under development in our laboratory.

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References