Activity of Phospholipase A Compared in Serum of Patients with Pancreatic and Nonpancreatic Diseases

D. Schmidt and G. E. Hoffmann

Phospholipase A (PLA) activity was measured with a semi-automated photometric test system that is based on liberation of fatty acids from phosphatidylcholine by phospholipases A₁ (EC 3.1.1.32) and A₂ (EC 3.1.1.4). We studied 528 serum samples from 86 patients whose lipase activities were increased owing to pancreatitis, pancreatic carcinoma, and extrapancreatic diseases. PLA activity showed no correlation with lipase or amylase activities or with the primary cause of the disease, but was clearly related to prognosis. Noncomplicated acute pancreatitis was characterized by “normal” PLA activities (0–10 U/L), whereas the values (50–137 U/L) were highest in necrotizing pancreatitis and sepsis with a lethal outcome. Changes in lipase and phospholipase A activities exhibited completely different time courses in the various diagnostic groups.

Additional Keyphrases: lipase • amylase • pancreatitis • prognosis • reference interval

Since the early observations of Zieve and Vogel (1) and Schmidt and Creutzfeld (2), phospholipase A (PLA) has been discussed as a pathogenic factor in acute pancreatitis (3, 4). More than two decades later, the question is still open as to whether serum PLA determinations really aid in accurate diagnosis of different forms of this disease. Some authors (5–7) reported that high activities of circulating PLA were correlated with severe necrotizing pancreatitis but not with the mild edematous form. Others (1, 8) found that PLA concentration changed more or less concomitantly with lipase and amylase and was not correlated with disease severity.

Recently, we developed a colorimetric procedure for determination of PLA activities in serum (9, 10); it is relatively easy to perform and, in contrast to conventional methods, can be automated (11) for routine clinical use. Here we report the results of our first clinical study with the new test, which we used to assay PLA in patients with increased lipase activities in serum.

Materials and Methods

We studied 528 serum samples from 86 patients in an intensive-care unit and a general gastroenterology ward. All sera were sent to the clinical laboratory for routine analysis and assayed without delay for lipase (EC 3.1.1.3) (12) and α-amylase (EC 3.2.1.1) (13) activities. Subsequently, the samples were stored at ~80°C for PLA determination within 24 months. During this period, freezing had no significant effect on PLA activities if the thawed serum was mixed carefully and any insoluble material was removed by recentrifugation.

All patients included in this study had at least one lipase value >200 U/L during their stay in the hospital. Diagnostic groups (see Results) were formed retrospectively according to clinical, intra-operative, sonographic, and radiological findings.

We measured PLA activities as described elsewhere (10). Briefly, the assay is based on liberation of fatty acid from 1 mL of an emulsion of soybean phosphatidylcholine by phospholipases A₁ (EC 3.1.1.32) and A₂ (EC 3.1.1.4) in a 0.1-mL serum sample at pH 8.0. The concentration of fatty acid is determined enzymatically as described previously for a lipase assay (14). In the present study we adapted the enzymatic determination of phosphatidylcholine-derived fatty acids to a selective analyzer (Hitachi 705; Boehringer Mannheim, F.R.G.).

Instrument settings were as follows: TEMP.: 38 °C; ASSAY CODE: END POINT; SAMPLE VOLUME: 20; R1 VOL.: 125 (S); R2 VOL.: 250-POSITION (S); WAVELENGTH 1: 660 NM; WAVELENGTH 2: 546 NM; RGT.BLK.CONC: 0; STD.CONC: 1000–1000-1; STD.ABS.ALLOWANCE: 10%

The assay was calibrated with a 1000 μmol/L oleic acid standard.

PLA activities were derived from the difference in fatty acid concentrations measured after 5 and 65 min of incubation. Intra-assay imprecision (CV, n = 5) was 10% at a mean value of 12 U/L and 3% at mean values of 31 and 52 U/L. Test response was linearly correlated with PLA activities up to 70 U/L.

Lipase and amylase activities were determined with commercial test kits from Boehringer Mannheim and Behringwerke, Marburg, F.R.G., respectively. According to the manufacturers’ inserts, normal values range up to 190 U/L for lipase and up to 66 U/L for amylase. The preliminary normal reference interval we determined for PLA was 0–10 U/L (10).

Results

Fifty-eight patients with pancreatic diseases (pancreatitis, pancreatic carcinoma) and 28 patients suffering primarily from extrapancreatic disorders were included in the study. Six of the latter group developed a marked lipaemia after endoscopic retrograde cholangio-pancreatography without exhibiting pathological findings in the pancreatic duct system. Others had increased lipase activities because of biliary and duodenal diseases, renal insufficiency, or shock. More details are given in Table 1.

Figure 1 shows typical examples of phospholipase values observed in pancreatic diseases. Figure 1A illustrates the enzyme activities in serum of a 53-year-old man who died from acute necrotizing pancreatitis on the eighth day after admission to the hospital. Although his lipase activity declined to 190 U/L within five days, PLA activities increased steadily, reaching 137 U/L just before his death.

In contrast, a 81-year-old man with an acute attack of noncomplicated pancreatitis showed only normal PLA activities (Figure 1B), whereas his lipase values were not clearly distinguishable from those depicted in Figure 1A.

A 42-year-old man with chronically relapsing pancreatitis...
showed increased values for both lipase and amylase and normal PLA activities during one month. Figure 1C depicts results obtained during the first 10 days of his hospital stay.

From these typical clinical examples and from correlation data reported previously (10) it is obvious that PLA is unrelated to lipase and amylase.

Figure 2 summarizes the maximum PLA activities measured in the individual course of each patient. Patients with acute pancreatitis (groups A–C) had normal activities of PLA only in noncomplicated edematous pancreatitis, whereas serum lipase reached its highest values (up to 24,000 U/L) in such cases. On the other hand, in all patients who died from necrotizing pancreatitis (group A) PLA values exceeded 50 U/L, whereas maximum lipase activities varied widely (250–8000 U/L).

Chronic pancreatitis was characterized by normal or slightly increased PLA values. Only two of 22 patients exhibited activities near 50 U/L. Both were admitted to the hospital with a serious and painful attack and a leukocyte count of more than 20,000/μL.

We also observed irregular increases in PLA activity in five patients with pancreatic cancer. One patient who died during his stay in the hospital had PLA values >50 U/L.

When we subdivided nonpancreatic diseases into lethal and nonlethal cases (Figure 2, groups F and G), we found above-normal PLA activities in the former group, especially in four patients who developed septicemia (maximum PLA values 37–134 U/L). All patients of group F died with signs of renal or respiratory insufficiency. Among the nonlethal cases (Figure 2G) only one patient, with myocardial infarction and septicemia, exhibited markedly increased PLA values (maximum 45 U/L). All other values measured in this group were <20 U/L.

In six cases with iatrogenic lipasemia after endoscopic

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**Table 1. Diagnostic Groups**

<table>
<thead>
<tr>
<th>Group</th>
<th>Diagnoses</th>
<th>No. patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Acute necrotizing pancreatitis (lethal)</td>
<td>7</td>
</tr>
<tr>
<td>B</td>
<td>Acute necrotizing pancreatitis (nonlethal)</td>
<td>11</td>
</tr>
<tr>
<td>C</td>
<td>Acute edematous pancreatitis (nonlethal)</td>
<td>11</td>
</tr>
<tr>
<td>D</td>
<td>Chronic pancreatitis (nonlethal)</td>
<td>24</td>
</tr>
<tr>
<td>E</td>
<td>Pancreatic carcinoma (1 lethal, 4 nonlethal)</td>
<td>5</td>
</tr>
<tr>
<td>F</td>
<td>Extrapancreatic diseases (lethal):</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- extrapancreatic neoplasms, mesenteric infarction, pneumonia, myocardial</td>
<td></td>
</tr>
<tr>
<td></td>
<td>infarction, chronic renal insufficiency, sepsis</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>Extrapancreatic diseases (nonlethal):</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- cholecystitis, cholelithiasis,</td>
<td></td>
</tr>
<tr>
<td></td>
<td>extrapancreatic neoplasms, liver cirrhosis, gastroenteritis, duodenal</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ulcer, myocardial infarction</td>
<td></td>
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</tbody>
</table>

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**Fig. 1. Typical values for lipase and phospholipase A activities in serum of patients with acute and chronic pancreatitis**


**Fig. 2. Maximum PLA activities observed in the course of different diseases**

Diagnostic groups A–G are defined in Table 1
retrograde cholangio-pancreatography, PLA activities never exceeded the normal range, although lipase reached values up to about 8000 U/L.

Irrespective of the diagnosis, the survival rate was negatively correlated with the peak activities of PLA in serum. Of those patients whose PLA values exceeded 50 U/L, 80% (eight of 10) died; of those with normal or slightly increased values, 95% survived (58 of 61 patients with all PLA values <20 U/L). No such correlations were found for lipase or amylace activities.

Discussion

Although the pathophysiological role of circulating PLA in the development of acute necrotizing pancreatitis has often been pointed out in reviews and handbooks, there is some disagreement among various reports. Our findings confirm the results of Schroeder et al. (8), Thuren et al. (6), and Büchler et al. (7) in that we observed a correlation between PLA activity and severity of pancreatitis.

However, we did not find serum PLA to be specific for pancreatic diseases, because markedly above-normal values were also found in severe septicemia of nonpancreatic origin. In 1984, Vadas (15), using a completely different analytical technique in which hydrolysis of E. coli phospholipids was measured, reported increased PLA activities in patients with septic shock.

Our results seem to disagree with the findings of Nevalainen et al. (6), who observed a strong correlation between amylace activities and the quantity of circulating PLA2 as measured immunochemically. This discrepancy between results by various methods was recently confirmed by Büchler et al. (7), who directly compared three PLA methods, including the present one and that of Nevalainen et al.

When one compares the various studies it seems reasonable to subdivide PLA tests into two categories: those methods yielding good correlations between phospholipase and other pancreatic enzymes seem to be specific for the pancreatic phospholipase A2, while the others, including the present assay, measure one or several other isoenzymes of phospholipase. Thus granulocyte and platelet PLA (16, 17) may contribute to the overall serum activity in severe pancreatic and nonpancreatic inflammatory diseases.

It can be assumed, on the other hand, that methods that measure PLA activity and yield a good correlation with the prognosis of pancreatitis and other severe diseases are likely to recognize the pathogenetic potency of the enzyme, while immunometric assays also include inactive proenzymes, enzyme–inhibitor complexes, or degradation products (8).

The new phospholipase assay (10) has to be subsumed to the second group of tests together with some fluorometric and radiochemical methods (5–7, 15) in that it predicts the lethal outcome of various diseases (see Results).

From the present data, the source of enzyme activity cannot be defined. Two preliminary experiments, however, indicate that it may be of extrapancreatic origin, even in necrotizing pancreatitis. Using our test system for an animal model of trypsin-induced pancreatitis (8), PLA activity was found to be increased in ascites fluid but not in serum. In human pancreatitis, serum PLA exhibited an alkaline rather than an acidic pH optimum (unpublished results), in contrast to the purified pancreatic enzyme (10). Further studies are presently being undertaken to elucidate the origin of PLA activities in serum in various diseases.

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References