SHORT COMMUNICATION

Serum Phospholipase A₂ in Patients after Splenectomy

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Summary: Phospholipase A₂ values increase in serum in various inflammatory states, infections, and postoperatively in surgical patients. Several organs, including the liver and spleen, have been suggested as sources of circulating phospholipase A₂. The purpose of the present work was to examine the possible role of the spleen as a source of elevated serum concentrations of phospholipase A₂ after surgery. Pre- and postoperative serum samples of patients undergoing splenectomy were studied for group I phospholipase A₂, group II phospholipase A₂, and C-reactive protein mass concentrations and catalytic activity concentration of phospholipase A₂. The catalytic activity concentration of phospholipase A₂ and the mass concentrations of group II phospholipase A₂ and C-reactive protein increased postoperatively (8.08 ± 1.40 U/l vs. 3.96 ± 0.89 U/l (mean ± SEM) for phospholipase A₂ catalytic concentration (p < 0.03), and 154.8 ± 32.1 μg/l vs. 47.5 ± 14.7 μg/l (mean ± SEM) for group II phospholipase A₂ mass concentration (p < 0.02, n = 7)). The mass concentration of group I phospholipase A₂ remained unchanged. The catalytic concentration of phospholipase A₂ correlated well with the mass concentration of group II phospholipase A₂ (p < 0.001, r = 0.846, n = 43). The concentration of C-reactive protein correlated well with the mass concentration of group II phospholipase A₂ (p < 0.001, r = 0.566, n = 43) in serum. The results indicate that group II phospholipase A₂ is released into the circulation after splenectomy, and the spleen seems not to be the source of circulating group II phospholipase A₂.

Introduction

Phospholipase A₂ catalyses the hydrolysis of the sn-2 fatty acyl bond of phospholipids and produces free fatty acids and lysophospholipids. The activation of phospholipase A₂ has been implicated as an early event in the inflammatory pathway (1). Mammalian secretory phospholipases A₂ are divided into two groups (2). Group I and group II phospholipases A₂ are M₉, 14000 enzymes that are structurally and immunologically distinct and are found in trace amounts in human serum (3). Group I phospholipase A₂ is a digestive enzyme secreted from pancreatic acinar cells into the lumen of the duodenum. Group II phospholipase A₂ is involved in the inflammatory reaction, but the exact function and cellular sources of this enzyme are still unknown (4).

The mass concentration of group II phospholipase A₂ increases in sera of patients with sepsis (5, 6), acute pancreatitis (7), postoperative states (8–10), and other severe acute diseases (11). The catalytically active enzyme circulating in blood in sepsis (3), acute pancreatitis (3), and after surgery (8–10) is group II phospholipase A₂. The mass concentration of group II phospholipase A₂ in serum changes in concert with C-reactive protein values, and it has been suggested that group II phospholipase A₂ is an acute phase protein (5, 8).

Materials and Methods

Patients

Seven consecutive patients undergoing splenectomy for haematological disease (n = 6) or traumatic rupture of the spleen (n = 1) in the University Central Hospital of Turku were enrolled in the present prospective study (see tab. 1 for diagnoses). The mean age of the patients was 57 years (range 29–67). There were five males and two females. Four of the patients suffered from a haematological malignancy, two had autoimmune idiopathic haemolytic anaemia, and one had traumatic rupture of the spleen. Only the patients without clinically documented infections or preoperatively elevated C-reactive protein values were included in the study. The investigation was approved by the local Ethical Committee.

Samples

In the patients with haematological disease, the serum samples were collected before operation (at 7.00 a.m. at the operation day) and thereafter daily (at 7.00 a.m.) until the sixth postoperative day. The serum sample from the patient with traumatic rupture was taken three hours before the spleen was removed. The samples were stored at −20 °C.

Phospholipase A₂ catalytic activity concentration

The catalytic activity concentration of phospholipase A₂ in serum was measured by incubating a 10 μl serum sample with 60 μmol 1,2-dipalmitoylphosphatidylcholine (Sigma, MO, St. Louis, USA) and 13.25 nmol 1-linoyleoyl-2-[14C]arachidonylphosphatidylethanolamine (DuPont, MA, NEN, USA) in mixed micelles for three hours at 40 °C (14). The release of radiolabelled free sn-2-fatty acid was detected with a RackBeta Liquid Scintillation Counter (Wallac, Turku, Finland), and the activity of the enzyme is expressed as units per litre (U/l, 1 U = 1 μmol liberated arachidonate per minute).

1) Enzymes:
Phospholipase A₂: 2-acylhydrolase (EC 3.1.1.4)
Newman-Keuls* multiple range test was used to test differences in the Student's t-test.

The results are expressed as mean ± standard error of the mean (SEM). The significance of the time-related changes was tested by the analysis of variance (ANOVA) with repeated measurements.

The mass concentrations of group I phospholipase A₂ (15), group II phospholipase A₂ (3) and C-reactive protein (16) were assayed in the serum samples by time-resolved fluoroimmunoassays as described earlier.

Statistical analysis
The results are expressed as mean ± standard error of the mean (SEM). The significance of the time-related changes was tested by the analysis of variance (ANOVA) with repeated measurements. Newman-Keuls' multiple range test was used to test differences between the preoperative and other values. Paired Student's t-test was used to compare the preoperative phospholipase A₂ activity and group II phospholipase A₂ mass concentrations with the maximal postoperative values. Pearson's linear correlation was used to study the correlations between the catalytic activity concentration of phospholipase A₂ and the mass concentrations of group I phospholipase A₂ and group II phospholipase A₂, and group II phospholipase A₂ and C-reactive protein.

Results
Clinical outcome of the patients
All the patients recovered uneventfully and none of them suffered from preoperative or postoperative complications. No signs of infection were observed.

Biochemical measurements
Figure 1 shows the phospholipase A₂ catalytic activity concentration, and the mass concentrations of group I phospholipase A₂, group II phospholipase A₂ and C-reactive protein in serum. The phospholipase A₂ catalytic activity concentration and the mass concentration of group II phospholipase A₂ increased after splenectomy. The maximum postoperative phospholipase A₂ catalytic activity concentrations and group II phospholipase A₂ mass concentrations were significantly higher than preoperative values (8.08 ± 1.40 U/l vs. 3.96 ± 0.89 U/l (mean ± SEM) for phospholipase A₂ catalytic activity concentration (p < 0.03), and 154.8 ± 32.1 μg/l vs. 47.5 ± 14.7 μg/l (mean ± SEM) for group II phospholipase A₂ mass concentration (p < 0.02)). Values returned to normal within the six days follow-up. The mass concentration of group I phospholipase A₂ did not change during the experiment. The concentration of C-reactive protein increased significantly after the operation; the highest values were observed at the third postoperative day (63.1 ± 15.1 mg/l vs. 6.09 ± 2.42 mg/l (mean ± SEM, p < 0.01)). The phospholipase A₂ catalytic activity concentration correlated well with the mass concentration of group II phospholipase A₂ (p < 0.001, r = 0.846, n = 43), but not with the mass concentration of group I phospholipase A₂ (p < 0.001, r = 0.566, n = 43). The concentration of C-reactive protein correlated well with the mass concentration of group II phospholipase A₂ (p < 0.001, r = 0.566, n = 43).

Discussion
Group II phospholipase A₂ has been found by immunohistochemistry in human (17) and rat (13) splenic macrophages. In the rat, increased phospholipase A₂ catalytic activity was found in splenic monocyte/macrophage and lymphocyte fractions and in the Kupffer cells of the liver after endotoxin administration. This catalytic ac-

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**Tab. 1** Clinical diagnosis and pathological findings in the current cases.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clinical diagnosis and stage</th>
<th>Haematological diagnoses and/or histopathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Autoimmune idiopathic haemolytic anaemia</td>
<td>Bone marrow: normal tissue morphology</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen: mild thrombocytopenia</td>
</tr>
<tr>
<td>2</td>
<td>Chronic lymphocytic lymphoma (low grade)</td>
<td>Bone marrow: normal tissue morphology</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen: Chronic lymphocytic lymphoma, diffuse type</td>
</tr>
<tr>
<td>3</td>
<td>Chronic lymphocytic leukaemia (low grade), autoimmune idiopathic haemolytic anaemia, erythropoiesis</td>
<td>Bone marrow: chronic lymphocytic leukaemia, autoimmune idiopathic haemolytic anaemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen: chronic lymphocytic leukaemia, extramedullary haematopoiesis, haemosiderosis</td>
</tr>
<tr>
<td>4</td>
<td>Rupture of the spleen</td>
<td>Bone marrow: normal tissue morphology</td>
</tr>
<tr>
<td>5</td>
<td>Chronic lymphocytic lymphoma (low grade)</td>
<td>Spleen: normal tissue morphology</td>
</tr>
<tr>
<td>6</td>
<td>Idiopathic thrombocytopenic purpura</td>
<td>Bone marrow: centroblastic/centrocytic follicular lymphoma</td>
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<tr>
<td></td>
<td></td>
<td>Spleen: centroblastic/centrocytic follicular lymphoma</td>
</tr>
<tr>
<td>7</td>
<td>Autoimmune idiopathic haemolytic anaemia, large cell anaplastic lymphoma (high grade)</td>
<td>Bone marrow: large cell anaplastic lymphoma, autoimmune idiopathic haemolytic anaemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen: large cell anaplastic lymphoma</td>
</tr>
</tbody>
</table>

Enzyme mass concentrations
The mass concentrations of group I phospholipase A₂ (15), group II phospholipase A₂ (3) and C-reactive protein (16) were assayed in the serum samples by time-resolved fluoroimmunoassays as described earlier.

**Fig. 1** The mass concentrations of group I phospholipase A₂ (hollow circles), group II phospholipase A₂ (filled circles) and C-reactive protein (hollow squares) and the phospholipase A₂ catalytic activity concentration (filled squares) before (day 0) and after splenectomy. The mass concentration of group II phospholipase A₂ and the catalytic activity concentration of phospholipase A₂ increases in concert with the concentration of C-reactive protein, while the mass concentration of group I phospholipase A₂ remains unchanged. Values are the mean ± SEM.
tivity was inhibited by an antibody against group II phospholipase A₂ (13). Enhanced expression of group II phospholipase A₂ mRNA was found after endotoxin challenge in rat spleen, aorta and thymus but not in the liver or kidney (12).

The liver has been proposed as a possible source of group II phospholipase A₂ by several authors. Endotoxin stimulation increased the mass concentration of group II phospholipase A₂ in liver perfusate (18), and group II phospholipase A₂ mRNA has been found in cultured hepatocytes (19). Moreover, mediators of the acute phase response including interleukin 1, interleukin 6 and tumour necrosis factor alpha, induced the expression and secretion of group II phospholipase A₂ in a human hepatoma cell line (20).

Splenectomy has a beneficial effect on the survival of patients with haematological malignancies (21). In contrast, splenectomy induces long-term disadvantages in the host-defence of the patients (22). Wiedermann and co-workers (23) studied acute phase protein profiles in hairy-cell leukaemia, and found that the occurrence of elevated C-reactive protein concentrations in serum was significantly higher in non-splenectomised than in splenectomised patients.

Elevated serum mass concentrations of C-reactive protein and phospholipase A₂ have been observed after surgery (8-10, 24), but, to our knowledge, early changes in acute phase protein concentrations after splenectomy have not been studied. In the current study, the catalytic activity concentration and the mass concentrations of both types of secretory phospholipases A₂ and the concentration of C-reactive protein were measured in serum after splenectomy to study whether the spleen is involved in the postoperative release of phospholipases A₂ into the circulation. We found postoperative increases in the catalytic activity concentration of phospholipase A₂ and the mass concentration of group II phospholipase A₂ in serum. The pre- and postoperative mass concentrations of group I phospholipase A₂ were below 10 μg/l which is regarded as the upper limit of the reference interval for group I phospholipase A₂ in serum (15). The upper limit of the reference interval for group II phospholipase A₂ in healthy individuals is 10.8 μg/l (3).

In the present study, the mean preoperative group II phospholipase A₂ value in serum was 47.5 ± 14.7 μg/l (mean ± SEM). Thus, the mean concentration is above the upper limit of the reference interval, and may be associated with the underlying haematological disease. In the patient with traumatic rupture of the spleen, the preoperative mass concentration of group II phospholipase A₂ in serum was 69.9 μg/l two hours after the estimated time of the rupture. This value was regarded as the preoperative mass concentration of group II phospholipase A₂.

The mass concentration of group II phospholipase A₂ increased in concert with the concentration of C-reactive protein, which supports the view that group II phospholipase A₂ represents an acute phase reactant. The serum phospholipase A₂ and C-reactive protein responses to splenectomy reported in the present study did not differ significantly from their responses to other operations, such as aortobifemoral reconstruction (9), and coronary artery bypass surgery (10). Thus, the spleen seems not to be involved in the postoperative release of group II phospholipase A₂ into the circulation.

In conclusion, the current results show that splenectomy does not prevent the release of group II phospholipase A₂ into the circulation after surgery. Other cellular sources, such as the liver, should be studied as a possible origin of circulating group II phospholipase A₂ during acute phase reaction.

Acknowledgments
The authors thank Heikki Peurauro, M.S., for valuable advice and Anne Jokilammi-Sillanen, M.S., for skilful technical assistance. Supported by The University of Turku Foundation, Sigrid Jusélius Foundation and The Academy of Finland Award 20751.

References


Received October 27, 1995/February 2, 1996
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