Cathepsin A activity of a parietal thrombus of an abdominal aortic aneurysm

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Abstract: We evaluated the cathepsin A activity of a parietal thrombus of an abdominal aortic aneurysm. We compared this activity to that of a retracted blood clot homogenate. Cathepsin A of aneurysm parietal thrombus homogenate and blood clot homogenate showed the highest activity on Z–Phe–Ala. It was lower on Z–Phe–Phe, Z–Glu–Tyr, Z–Glu–Phe, Z–Gly–Phe, and the lowest activity was on Z–Gly–Ala. We conclude that cathepsin A’s activity on a parietal thrombus of an aneurysm is much higher than blood clot cathepsin A activity. (Folia Histochemica et Cytobiologica 2011; Vol. 49, No. 1, pp. 10–12)

Key words: parietal thrombus of abdominal aortic aneurysm, blood clots, cathepsin A

Introduction

An aneurysm comprises a widening of the arterial lumen by at least 50%. Aneurysms are caused by atherosclerosis and inflammatory changes as well as enzymatic and immunological processes occurring in the arterial wall [1–3].

Aneurysmal widening of the abdominal aorta is most frequently filled with thrombocytic parietal thrombi, the formation of which brings about vascular endothelium damage and disturbs laminar blood flow [4, 5]. It is followed by the adhesion, activation and aggregation of blood platelets, and the platelet plug is strengthened with fibrin fibers [6, 7]. Neutrophils, monocytes, lymphocytes, fibroblasts, and endothelial cells penetrate the aneurysmal thrombus [8, 9]. The thrombus rebuilding and its influence on the aneurysmal wall depend mainly on the activity of the proteolytic enzymes that are present [5, 10].

The aim of our study was to evaluate the specificity and activity of cathepsin A (EC 3.4.16.1) of the parietal thrombus of an abdominal aortic aneurysm. We compared this activity to that of a retracted blood clot.

Material and methods

Parietal thrombi were collected from 15 patients (14 men and one woman, aged 65 ± 5 years) operated on for aortic aneurysm in the Department of Vascular Surgery and Transplantology of the Medical University of Bialystok. The aneurysms were localized below renal branching-off. Retracted blood clots were obtained from the same patients before the operations. The thrombi and the blood clots were stored at −75°C until they were examined. The whole 10% homogenate of thrombi and blood clots was prepared in 0.15 mol/l of KCl using a knife homogenizer, type Politron, and filtrated through polyamide fabric [11]. Z–Phe–Ala, Z–Phe–Phe, Z–Glu–Tyr, Z–Glu–Phe, Z–Gly–Phe, and Z–Gly–Ala, (Sigma–Aldrich, USA), were used to determine cathepsin A activity in homogenates [12, 13]. Sodium-acetic acid buffer in the amount of 0.1 ml 0.2 mol/l was added to 0.3 ml of homogenate and, after adding 0.1 ml 30 mmol/l of the substrate dissolved in dimethyl sulfoxide, it was incubated at 37°C for four hours. Both homogenate and buffer had a pH of 5.0. The reaction was interrupted by adding 0.5 ml 10% trichloroacetic acid. The samples precipitated at time zero were the controls. The amount of nmols of released C-end amino acid was determined in suprasedimentary fluid obtained through centrifuging, using the ninhydrinic method [14]. The results were expressed as tissue grams and incubation time, as well as protein mg and DNA µm.
The Bradford method was used to determine protein content [15] and DNA content was determined using the Burton method [16]. The results were statistically analyzed using a U Mann–Whitney test, assuming p < 0.05 as statistically significant.

### Results and discussion

Cathepsin A of an aneurysm parietal thrombus homogenate and a blood clot homogenate, expressed as tissue gram and one hour of incubation, showed the highest activity on hydrophobic-hydrophilic substrate Z–Phe–Ala (Table 1). The amounts were 18.2 Ala nmol/g/h for the thrombus, and 8.2 Ala nmol/g/h for the blood clot. The effect on hydrophobic-hydrophobic substrate Z–Phe–Phe was lower. Next was the effect on hydrophilic-hydrophobic substrate Z–Glu–Tyr, Z–Glu–Phe, Z–Gly–Phe. The lowest effect was on hydrophilic-hydrophilic substrate Z–Gly–Ala. The activity of cathepsin A of an aneurysm parietal thrombus homogenate on examined substrates was approximately 1.5 to 2.2 times higher than the activity of cathepsin A on blood clot homogenate. The differences were statistically significant in most substrates. In the case of Z–Glu–Tyr, they were at the borderline of statistical significance. In the case of Z–Gly–Ala they were statistically insignificant.

### Table 1. Cathepsin A activity of a parietal thrombus of an abdominal aortic aneurysm and blood clots determined on Z-dipeptides (at pH 5.0)

<table>
<thead>
<tr>
<th>Substrates</th>
<th>C-terminal amino acid [nmol/g/h]</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parietal thrombus</td>
<td>Blood clot</td>
</tr>
<tr>
<td>Z–Phe–Ala</td>
<td>18.2 ± 1.72</td>
<td>8.2 ± 0.63</td>
</tr>
<tr>
<td>Z–Phe–Phe</td>
<td>6.4 ± 0.54</td>
<td>2.7 ± 0.26</td>
</tr>
<tr>
<td>Z–Glu–Tyr</td>
<td>2.3 ± 0.19</td>
<td>1.2 ± 0.11</td>
</tr>
<tr>
<td>Z–Glu–Phe</td>
<td>1.6 ± 0.14</td>
<td>1.1 ± 0.09</td>
</tr>
<tr>
<td>Z–Gly–Phe</td>
<td>1.4 ± 0.12</td>
<td>1.0 ± 0.08</td>
</tr>
<tr>
<td>Z–Gly–Ala</td>
<td>0.6 ± 0.07</td>
<td>0.4 ± 0.05</td>
</tr>
</tbody>
</table>

### Table 2. Protein and DNA contents and cathepsin A activity of a parietal thrombus and a blood clot expressed as protein milligrams and DNA micrograms

<table>
<thead>
<tr>
<th>Determination</th>
<th>Parietal thrombus</th>
<th>Blood clot</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein, mg/g tissue</td>
<td>38.2 ± 2.92</td>
<td>58.6 ± 5.65</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>DNA, µg/g tissue</td>
<td>125.5 ± 13.84</td>
<td>52.6 ± 6.32</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Cathepsin A*, Ala nmol/mg protein</td>
<td>0.476 ± 0.06</td>
<td>0.140 ± 0.01</td>
<td>p = 0.029</td>
</tr>
<tr>
<td>Cathepsin A*, Ala nmol/µg DNA</td>
<td>0.145 ± 0.02</td>
<td>0.156 ± 0.02</td>
<td>p = 0.26</td>
</tr>
</tbody>
</table>

*Substrate: Z–Phe–Ala

Protein content in thrombus homogenate was 38.2 mg/g of tissue, while in blood clot homogenate it was 58.6 mg/g of tissue (Table 2). Cathepsin A activity, determined using Z–Phe–Ala expressed as protein, was higher in thrombus homogenate (0.476 Ala nmol/protein mg) than in blood clot homogenate (0.140 Ala nmol/protein mg).

DNA content in the thrombus was 125.5 µg/tissue g. In the blood clot, it was 52.6 µg/tissue g. Cathepsin A activity, determined using Z–Phe–Ala expressed as DNA content, was slightly lower in the thrombus (0.145 Ala nmol/µg DNA) than in the blood clot (0.156 Ala nmol/µg DNA).

On the other hand, expressed as protein content, it was higher in the thrombus homogenate (0.476 Ala nmol/g protein) than in the blood clot homogenate (0.140 Ala nmol/g protein). These differences, apart from the last one, were statistically significant. They resulted from large amounts of blood platelets that contain cathepsin A and do not contain DNA in the thrombus, and large amounts of erythrocytes that do not contain either cathepsin A or DNA in the blood clot [17–19].

Cathepsin A is a lysosomal carboxypeptidase. It splits C-end amino-acids from peptides and proteins, especially quickly if phenylalanine residue occurs in the penultimate position, in a weakly acidic environment [12, 19–21]. Our results are in accord with this.
Cathepsin A participates in protein degradation and activates/inactivates peptic hormones and biologically active peptides [20, 22]. This enzyme has no endogenous inhibitors [23] and its peptidizing activity depends on the availability of the substrate, and a pH of between 4.5 and 5.5 [20, 23]. In a pH of 7.0–7.5, cathepsin A shows amidazing and esterazing activity [22, 24]. It also plays a role as a protective protein to certain glycosidases [25, 26]. These activities of cathepsin A may also apply to the parietal thrombus of an aortic aneurysm.

References


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