FOLIA HISTOCHEMICA
ET CYTOBIOLOGICA
Vol. 49, No. 1, 2011
pp. 10–12

REVIEW

VIA MEDICA

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

Marta Siergiejuk, Marek Gacko, Anna Worowska

Department of Vascular Surgery and Transplantology, Medical University of Bialystok, Poland

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.

Correspondence address: M. Siergiejuk, Dept. of Vascular Surgery and Transplantology, Medical University of Bialystok, M. Skłodowskiej-Curie Str. 24a, 15–276 Bialystok, Poland; tel.: (+ 48 85) 746 82 77; fax: (+ 48 85) 746 88 96; e-mail: martasiergiejuk1983@o2.pl

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 ninhydrynic method [14]. The results were expressed as tissue grams and incubation time, as well as protein mg and DNA μm.

©Polish Society for Histochemistry and Cytochemistry
Folia Histochem Cytobiol. 2011
10.5603/FHC.2011.0003

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

Substrates	C-terminal amino acid [nmol/g/h]		Statistical significance
	Parietal thrombus	Blood clot	
Z–Phe–Ala	18.2 ± 1.72	8.2 ± 0.63	$p < 0.001$
Z–Phe–Phe	6.4 ± 0.54	2.7 ± 0.26	$p = 0.05$
Z–Glu–Tyr	2.3 ± 0.19	1.2 ± 0.11	$p = 0.058$
Z–Glu–Phe	1.6 ± 0.14	1.1 ± 0.09	$p = 0.049$
Z–Gly–Phe	1.4 ± 0.12	1.0 ± 0.08	$p = 0.032$
Z–Gly–Ala	0.6 ± 0.07	0.4 ± 0.05	$p = 0.45$

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

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

*Substrate: Z–Phe–Ala

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 hydrophobie-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 hydrophobie-hydrophobic substrate Z–Phe–Phe was lower. Next was the effect on hydrophilo-hydrophobic substrate Z–Glu–Tyr, Z–Glu–Phe, Z–Gly–Phe. The lowest effect was on hydrophilo-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.

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 phenyloalanine residue occurs in the penultimate position, in a weakly acidic environment [12, 19–21]. Our results are in accord with this.

©Polish Society for Histochemistry and Cytochemistry

10.5603/FHC.2011.0003

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

1. Ailawadi G, Eliason JL, Upchurch GR. Current concepts in the pathogenesis of abdominal aortic aneurysm. *J Vasc Surg.* 2003;38:584–588.
2. Gacko M. Role of lysosomal proteolytic enzymes in pathogenesis of aortic aneurysm. *Pol Przegl Chir.* 2000;72:806–814.
3. Rizas KD, Ippagunta N, Tilson MD. Immune cells and molecular mediators in the pathogenesis of the abdominal aortic aneurysm. *Cardiol Rev.* 2009;17:201–210.
4. Arita T, Matsunaga N, Mita T et al. Spared flow tract within the mural thrombus of an aortic aneurysm: its pathogenesis and clinical importance. *J Comput Assist Tomogr.* 2000;24:86–91.
5. Vorp DA, Lee PC, Wang DH et al. Association of intraluminal thrombus in abdominal aortic aneurysm with local hypoxia and wall weakening. *J Vasc Surg.* 2001;34:291–299.
6. Cnotliwy M, Jastrzębska M, Wiernicki I et al. Coagulation and fibrinolysis in the abluminal layer of the thrombus within abdominal aortic aneurysm. *Acta Angiol.* 2007;13: 114–121.
7. Gacko M, Worowska A, Głowiński S. Coagulative and fibrinolytic activity in parietal thrombus of aortic aneurysm. *Ann Acad Med Biolostociensis.* 1999;44 :102–110.
8. Adolph R, Vorp DA, Steed DL et al. Cellular content and permeability of intraluminal thrombus in abdominal aortic aneurysm. *J Vasc Surg.* 1997;5:916–926.
9. Kirchhofer D, Riederer MA, Baumgartner HR. Specific accumulation of circulating monocytes and polymorphonuclear leukocytes on platelet thrombi in a vascular injury model. *Blood.* 1997;89:1270–1278.
10. Gacko M, Worowska A, Głowiński S. Cathepsin D and cathepsin L in the wall of aortic aneurysm and parietal thrombus determined by means of synthetic substrates. *Bull Pol Acad Sci.* 2000;48:11–15.
11. Karwowska A, Gacko M, Worowska A et al. Próba standaryzacji rozdrabniania tkanek zwierzęcych do badań analitycznych. *Bromat Chem Toksykol.* 2006;38:199–202.
12. Kawamura Y, Matoba T, Hata T et al. Purification and some properties of cathepsin A of large molecular size from pig kidney. *J Biochem.* 1974;76:915–924.
13. Siergiejuk M. Katepsyna A skrzepliny przyściennej tętniaka aorty brzusznej. *Med Biol Sci.* 2009;23:49.
14. Moore S. Amino acid analysis: aqueous dimethyl sulfoxide as solvent for the ninhydrin reaction. *J Biol Chem.* 1968;243:6281–6283.
15. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976;72:248–254.
16. Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J.* 1956;62:315–323.
17. Beese J, Farr W, Grüner E, Haschen RJ. Proteolytische Enzyme in normalen menschlichen Blutplättchen. *Klin Wschr.* 1966;44:1051–1053.
18. Haschen RJ. *Proteolytic enzymes in blood cells. Intracellular protein catabolism.* Hanson H, Bohley P (ed.). Wissenschaftliche Beitrage der Martin-Luther-Universitat Halle-Wittenberg, Halle: Seale; 1973;398–408.
19. Ostrowska H, Wójcik C, Worowski K. Cathepsin A and chymotrypsin-like activity of the proteasome in human blood platelets. *Folia Histochem Cytobiol.* 1999;7:153–154.
20. Gacko M, Worowska A, Woźniak A et al. Lizosomalna karboksypeptydaza A. *Post Bioch.* 2005;51:162–170.
21. Kawamura Y, Matoba T, Dio E. Submit structure of pig kidney cathepsin A. *J Biochem.* 1980;88:1159–1161.
22. Jackman HL, Tan FL, Tamei H et al. A peptidase in human platelets that deamidates tachykinins. *J Biol Chem.* 1990;265:11265–11272.
23. Pshezhetsky AV. Lysosomal carboxypeptidase A. In: Barrett AJ, Rawlings ND, Woessner JF (ed.). *Handbook of Proteolytic Enzymes*, Vol 2. Academic Press, San Diego 2004; 1923–1929.
24. Jackman HL, Morris PW, Deddish PA et al. Inactivation of endothelin I by deamidase (lysosomal protective protein). *J Biol Chem.* 1992;267:2872–2875.
25. Cuervo AM, Mann L, Bonten EJ et al. Cathepsin A regulates chaperone-mediated autophagy through cleavage of the lysosomal receptor. *EMBO J.* 2003;22:47–59.
26. Pshezhetsky AV, Ashmarina M. Lysosomal multienzyme complex: biochemistry, genetics and molecular pathophysiology. *Prog Nucleic Acid Res Mol Biol.* 2001;69:81–114.

Submitted: 27 September, 2010
Accepted after reviews: 13 January, 2011

©Polish Society for Histochemistry and Cytochemistry