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# Effect of heavy metal cations on the activity of cathepsin D (*in vitro* study)

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**Abstract:** We studied the effect of heavy metal cations: $Fe^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Hg^{2+}$, $Pb^{2+}$ on the activity of cathepsin D in human aorta homogenate and blood serum. The concentration of cations was 1 mmol/l. Hemoglobin was the cathepsin D substrate. The activity of cathepsin D was determined at pH 3.5. Only $Hg^{2+}$ cations inhibit the activity of cathepsin D. Cations $Hg^{2+}$ damage lysosomes and release cathepsin D from these organelles. *(Folia Histochemica et Cytobiologica 2012, Vol. 50, No. 3, 432–435)*

**Key words**: cathepsin D, lysosomes, heavy metals

## Introduction

Cations, $Fe^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ are natural components of the organism, while $Cd^{2+}$, $Hg^{2+}$ and $Pb^{2+}$ can only be found in a state of intoxication. Whichever pathway heavy metals take to reach the body, they pass through the blood and come into contact with plasma proteins, morphotic components and vascular endothelial cells [1–3]. In arterial disorders, changes in the content of these metal cations have been observed both in plasma and in vascular wall lesions [4–10]. Agarwal et al. found that high levels of serum cadmium are associated with cardiovascular and cerebrovascular disease [11]. Poręba et al. observed that exposure to lead can be associated with increased blood pressure and accelerated progression of atherosclerosis [12]. Moreover, they found that higher blood concentrations of lead and cadmium are independent risk factors for the incidence of arterial hypertension in subjects chronically exposed to heavy metals [13, 14]. Other researchers have claimed that mercury can also be a risk factor for hypertension and atherosclerosis [15]. In hypertension, atherosclerosis and aneurysm, changes in the activities of lysosomal proteolytic enzymes, e.g. cathepsin D, occur in blood plasma and arterial walls [16–20].

The study objective was to determine the effect of heavy metal cations of variable ionic radius ($Fe^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Hg^{2+}$, $Pb^{2+}$) on the activities of cathepsin D in human aorta homogenate and blood serum, and on their release from liver lysosomes.

## Material and methods

Hemoglobin, (Difco Laboratories, USA); Folin-Ciocalteu reagent, (Merck, Germany); Bradford reagent and trichloracetate acid, (Sigma, USA); biuret reagent and metal cations (chlorides): $Fe^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Hg^{2+}$, $Pb^{2+}$, (POCh, Gliwice, Poland).

The whole abdominal aorta homogenate from ten organ donors was prepared according to the method described previously [21]. Serum was obtained from ulnar vein blood collected from ten healthy subjects. The blood was incubated for 1 h at 37°C and centrifuged (1,500 × g, 30 min., 2°C).

Metal cations solutions (0.05 ml) at a concentration 10.0 mmol/l (in control 0.15 mol/l KCl for homogenate and 0.15 mol/l NaCl for serum) was added to 0.35 ml homogenate and 0.35 ml serum, pH 7.5. Following a 30-min preincu-

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bation at 37°C, the samples were adjusted to pH 3.5. The pH 3.5 samples were supplied with 0.1ml of 6% hemoglobin (pH 3.5) and incubated at 37°C for 6 hours (both homogenate and serum). The reaction was discontinued by adding 0.1 ml of 10% trichloracetic acid. Precipitated samples at time 0 were treated as control. In the supernatant obtained through centrifugation, the amount of released tyrosine was determined [22].

Lysosomes-containing homogenate of six rabbit livers was prepared following the method described previously [23]. A 0.15 ml 8.0 mmol/l cation solution (in control 0.15 mol/l KCl), pH 7.5, was added to 1.05 ml of this homogenate (pH 7.5), and incubated for 30 min. at 37°C. When pH 5.0 was obtained, the samples were centrifuged (1,500 × g, 30 min., 2°C). The obtained cytosol was adjusted to pH 3.5, and the activities of cathepsin D were determined as described above. Their activities were also investigated in whole liver homogenate [21].

The activity of cathepsin D was expressed as the level of released tyrosine (nmol) per one gram of tissue and per hour in homogenate and cytosol, and per milliliter and per hour in serum.

The Bradford method was used to determine protein in homogenate and cytosol [24]. Serum protein was determined using the biuretic method [25].

## Results

Only $Hg^{2+}$ cations inhibited the activities of cathepsin D in the aorta homogenate (Table 1). The mean protein content in the aorta homogenate was 12.7 mg/g tissue.

$Hg^{2+}$ cations also inhibited the activity of cathepsin D in blood serum (Table 2). The mean serum protein was 72.4 mg/ml.

The $Hg^{2+}$ cation released the highest amounts of cathepsin D from hepatocyte lysosomes (Table 3). The release of this cathepsin was accompanied by a slight release of protein.

## Discussion

Divalent metal cations bind to the carboxyl, amino, disulphide and thiol groups of proteins [26–29]. With carboxyl groups, they form salt-type bonds, with ami-

**Table 1.** Influence of heavy metals cations on activity of aorta homogenate cathepsin D

| Cation [1 mmol/l] | Cathepsin D | |
|---|---|---|
| | Tyr [nmol/g/h] | Inhibition (%) |
| $Fe^{2+}$ | 709.2 ± 70.2 | 3.9 |
| $Cu^{2+}$ | 710.5 ± 71.8 | 3.8 |
| $Zn^{2+}$ | 724.3 ± 72.3 | 1.8 |
| $Cd^{2+}$ | 715.2 ± 72.3 | 2.9 |
| $Hg^{2+}$ | 629.8 ± 69.3* | 14.6 |
| $Pb^{2+}$ | 728.6 ± 71.0 | 1.2 |
| KCl, 0.15 mol/l (control) | 737.2 ± 73.0 | 0.0 |

*$p < 0.05$ compared to the control

**Table 2.** Influence of heavy metals cations on activity of serum cathepsin D

| Cation [1 mmol/l] | Cathepsin D | |
|---|---|---|
| | Tyr [nmol/g/h] | Inhibition (%) |
| $Fe^{2+}$ | 47.3 ± 4.8 | 8.9 |
| $Cu^{2+}$ | 48.5 ± 4.9 | 6.5 |
| $Zn^{2+}$ | 51.7 ± 5.4 | 0.4 |
| $Cd^{2+}$ | 50.2 ± 5.3 | 3.3 |
| $Hg^{2+}$ | 37.7 ± 3.9* | 27.4 |
| $Pb^{2+}$ | 51.0 ± 5.6 | 1.7 |
| NaCl, 0.15 mol/l (control) | 51.9 ± 5.2 | 0.0 |

*$p < 0.05$ compared to the control

**Table 3**. Influence of heavy metals cations on the release of cathepsin D from lysosomes of liver

| Cation [1 mmol/l] | Cathepsin D | | Protein [mg/g], (%) | |
|---|---|---|---|---|
| | Tyr [nmol/g/h] | Release (%) | [mg/g] | Release (%) |
| $Fe^{2+}$ | 86.6 ± 8.2 | 12.7 | 2.4 ± 0.2 | 6.3 |
| $Cu^{2+}$ | 89.5 ± 8.4 | 13.3 | 2.5 ± 0.2 | 6.5 |
| $Zn^{2+}$ | 84.2 ± 8.3 | 12.5 | 2.6 ± 0.3 | 6.9 |
| $Cd^{2+}$ | 87.6 ± 7.9 | 12.9 | 2.5 ± 0.3 | 6.7 |
| $Hg^{2+}$ | 98.8 ± 8.9* | 14.9 | 2.2 ± 0.2 | 5.9 |
| $Pb^{2+}$ | 77.7 ± 8.0 | 11.6 | 2.7 ± 0.2 | 7.1 |
| KCl, 0.15 mol/l (control) | 80.9 ± 7.6 | 12.0 | 2.7 ± 0.3 | 7.1 |
| Whole homogenate | 674.2 ± 65.8 | 100.0 | 38.0 ± 4.1 | 100.0 |

*$p < 0.05$ compared to the control

no and disulphide groups they form complex bonds, and with thiol groups they form sulphides.

Binding of metal cations to carboxyl groups of the Asp33 and Asp231 residues of the catalytic site of cathepsin D plays a major role in the inhibition of its activity [30]. This refers mainly to the mercuric cation, which shows strong affinity for carboxyl groups.

The affinity of metal cations for proteins depends on the dimension of their ionic radius and standard potential [31]. The $Hg^{2+}$ cation, with the ionic radius of 102 pm and $Hg^{2+}/Hg$ standard potential of +0.851 V, exhibits the strongest inhibitory effect on cathepsin D [32]. Cations with a greater or smaller ionic radius and higher and lower standard potential do not inhibit cathepsin D activity.

Increased permeability of the lysosomal membranes is caused by damage to the structure of membrane lipids and proteins directly by metal cations and reactive oxygen species formed through the action of these cations [3, 33–39]. This leads to cathepsin release from lysosomes. However, the activity of cathepsin D secreted to cytosole is reduced by the part inactivated by the respective cations.

Specific binding proteins (metallothionein, transferrin, ferratin, ceruloplasmin and others) prevent the toxic action of heavy metal cations [40–42]. The metal binding exogenous compounds include ethylenodiaminotetraacetate acid, diethylenotriaminopentaacetic acid, N-acetylocystein, penicillamine and defероxamine [43–45]. The concentrations of heavy metal cations used in the current experiment resembled those in the blood and tissues of intoxicated subjects [46]. Their *in vitro* effects do not differ from the ones observed in the acute intoxication of laboratory animals, in which cathepsin D activity was reduced both in blood plasma and in organs [34, 47, 48]. However, no such relationship was found in the wall of atherosclerotic or aneurysmatic arteries [6, 17, 49, 50].

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