ORIGINAL PAPERS

Adv Clin Exp Med 2011, **20**, 3, 255–261
ISSN 1230-025X

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Assessment of Glucose-6-Phosphatase Activity in the Submandibular Salivary Gland of Rats Exposed to Cadmium and/or Zinc in Drinking Water

Ocena aktywności glukozo-6-fosfatazy w śliniance podżuchwowej szczura eksponowanego na kadm i /lub cynk z wodą do picia

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Abstract

Background. Analyzing glucose-6-phosphatase activity (G-6-P-aza) in histochemical investigations allows the symptoms of metal intoxication in animal cells to be assessed.
Objectives. Assessment of the enzyme marking the activity of the endoplasmic reticulum involved in the production of proteins in the cells of the submandibular salivary gland of rats exposed to cadmium and /or zinc in their drinking water.
Material and Methods. The study used 144 male Wistar rats aged 5–6 weeks, with an initial body weight of 189–200 g. Cadmium was administered in aqueous solutions of cadmium chloride ($CdCl_2$) at concentrations of 5 mg Cd/dm^3 or 50 mg Cd/dm^3. Zinc was administered in the form of zinc chloride ($ZnCl_2$) in aqueous solutions at concentrations of 30mg Zn/dm^3 or 60mg Zn/dm^3. The animals were exposed to the metals for periods of 6 months (series I) and 12 months (series II). The dissected submandibular salivary glands were used as material for analysis. The activity of glucose-6-phosphatase (G-6-P-ase) (E.C.3.1.3.9) was assessed using the Wachstein-Meisel method with glucose-6-phosphate sodium salt (G-6-P) (Sigma).
Results. In most of the experimental groups, the serous acinar cells of the salivary gland showed an enhanced reaction to glucose-6-phosphatase as compared to the control animals.
Conclusions. The toxic actions of cadmium and zinc led to some changes in the activity of glucose-6-phosphatase in the cells of the submandibular salivary gland (**Adv Clin Exp Med 2011, 20, 3, 255–261**).

Key words: cadmium, zinc, glucose-6-phosphatase, submandibular salivary gland, rat.

Streszczenie

Wprowadzenie. Analiza aktywności glukozo-6-fosfatazy (G-6-P-aza) w badaniach histochemicznych umożliwia ocenę wystąpienia objawów zatrucia metalami w obrazie komórki zwierzęcej.
Cel pracy. Ocena aktywności enzymu markującego aktywność siateczki śródplazmatycznej uczestniczącej w wytwarzaniu białek w komórkach ślinianki podżuchwowej szczurów, eksponowanych na kadm i/lub cynk z wodą do picia.
Materiał i metody. Badania przeprowadzono na 144 szczurach, samcach rasy Wistar w wieku 5–6 tygodni o początkowej masie ciała 180–200 g. Kadm podawano w wodnych roztworach chlorku kadmu ($CdCl_2$) w stężeniu 5 mg Cd/dm^3 lub 50 mg Cd/dm^3. Cynk zwierzęta otrzymywały w postaci chlorku cynku ($ZnCl_2$), również w wodnych roztworach w stężeniu 30 mg Zn/dm^3 i 60 mg Zn/dm^3. Zwierzęta były eksponowane na badane metale przez okres 6 (seria I) i 12 miesięcy (seria II). Materiał do badań histoenzymatycznych stanowiły wypreparowane ślinianki podżuchwowe. Aktywność glukozo-6-fosfatazy (G-6-P-aza) (E.C.3.1.3.9) badano według Wachstein-Meisel z użyciem soli sodowej G-6-P firmy Sigma.
Wyniki. W pęcherzykach surowiczych ślinianki nastąpiło nasilenie reakcji na glukozo-6-fosfatazę w większości grup doświadczalnych w porównaniu z grupą kontrolną.
Wnioski. W odpowiedzi na toksyczne działanie kadmu i cynku dochodziło do zmiany aktywności glukozo-6-fosfatazy w komórkach ślinianki podżuchwowej (**Adv Clin Exp Med 2011, 20, 3, 255–261**).

Słowa kluczowe: kadm, cynk, glukozo-6-fosfataza, ślinianka podżuchwowa, szczur.

Cadmium is a major environmental pollutant and its accumulation in the organism leads to systemic and organ dysfunction. In such target organs as the liver or kidney, cadmium ions combine with metallothionein to form cadmium-thionein. The process of cadmium binding to metallothionein entails changes in zinc and copper distribution, and the toxic action of cadmium results from its effect on these two bioelements [1]. A dose- and exposure time-dependent increase in cadmium accumulation, which may occur with the involvement of metallothionein, has been described in a previous article [2].

Cadmium released from natural and industrial sources is incorporated into the food chain and is the main source of humans' environmental exposure to cadmium intoxication [3]. Smoking is another important source of exposure to this element [4]. The mechanism of cadmium transport in the human body from the site of absorption to the internal organs has not been elucidated yet [5]. It is, however, believed that in humans, as in animals, after cadmium has been absorbed into the blood, it binds to albumin to form a cadmium-albumin complex. When this reaches the liver it undergoes disintegration and the free Cd^{2+} ions that are produced induce synthesis of metallothionein (MT).

Metallothionein is a low-molecular protein that plays a key role in the metabolism of cadmium and other heavy metals, taking part in their absorption, transport, distribution and detoxication [6–9]. In the liver, cadmium ions and metallothionein form cadmium-thionein complexes. Some of these complexes are released into the plasma; from there they are transported to the kidneys, where they undergo filtration in the renal glomeruli [9]. The filtered complex disintegrates in the lumen of the glomeruli or is reabsorbed by cells of the proximal canaliculi. In the lysosomes of these cells, the complex undergoes dissociation and cadmium ions are released from the protein-bond. Friedman and Brzóska [10, 11] have found that cadmium is also reabsorbed by epithelial cells of the distal canaliculi, where it induces generation of the Cd-MT complex, thus enhancing its accumulation in the kidney [12]. Cadmium can also be accumulated in other soft tissues, including the submandibular salivary gland, in which it can induce MT production.

Despite numerous studies, the mechanism of cadmium's toxic action has not been fully elucidated yet, but it results from the blocking of important metabolic pathways via the inactivation of certain enzymes and is based on the displacement/replacement of metal ions in cytosolic and mitochondrial metalloenzymes by cadmium ions, which show a greater affinity for sulfhydryl groups (-SH) than other metals.

The inactivation of such enzymes as α-1-antitripsin, succinate dehydrogenase, oxidase, phosphatase and ATP involved in the metabolism of erythrocytes has a harmful effect on the hemopoietic system [13, 14]. Moreover, cadmium competing with other metals for the active centers in metalloenzymes also inhibits protein synthesis, energetic processes, membrane transport, and the metabolism of lipids and nucleic acids [14]. Cadmium in the cell binds to the proteins of the cytoplasm, the mitochondrial and lysosomal membranes and to the proteins of cell nuclear membranes. At the cellular level, mitochondria are the target site for the toxic action of cadmium [15]. Cadmium reduces the activity of the main mitochondrial enzymes, i.e. succinate dehydrogenase and cytochrome oxidase, and at the same time disturbs the phosphorylation processes in the mitochondrial membranes [16]. As shown by Martel [17], the inhibition of energetic processes in mitochondria occurs even in the initial phases of exposure, even at low doses of cadmium.

Damage to the antioxidative barrier and enhancement of oxidative stress are further very important mechanisms of the toxic action of cadmium, resulting in the production of free superoxide radicals, membrane lipid peroxidation [13,18,19] and negative effects on the enzymatic antioxidants: superoxide dysmutase, catalase, glutathione peroxidase and reductase as well as non-enzymatic antioxidants, such as vitamin E, vitamin C, glutathione, carotenes and retinols [20].

Systemic zinc homeostasis involves metallothioneins, i.e. zinc-binding proteins, mostly found in the intestinal mucosa, in the liver and in the kidneys [1]. Zinc (like cadmium, mercury and copper) induces MT synthesis, forming zinc-protein complexes with it, mainly in the liver.

Like copper, magnesium and iron, zinc is an indispensable element for the normal course of many metabolic processes in the body. A review of the literature shows that these bioelements have been the subject of numerous research studies due to their involvement in many key enzymatic transformations [21].

Zinc is present in approximately 80 enzymes (oxydoreductases, transferases and hydrolases), which indicates its multidirectional biochemical activity [21].

Zinc changes cadmium distribution in the body, increasing its accumulation in the liver and kidneys, but decreasing it in other tissues [22]. Carrier proteins such as transferrin (which binds iron and zinc) or ceruloplasmin (which binds copper) may also accumulate cadmium. A study conducted on rats showed that an increased zinc supply elevates the level of metallothionein in the

small intestine and decreases cadmium absorption in the body [1]. The attention many researchers are giving to interactions between cadmium and bioelements may in the future lead to the use of certain bioelements for screening tests in order to assess preventive actions or to test for exposure to heavy metals, including cadmium toxicity.

Analyzing glucose-6-phosphatase activity (G-6-P-ase) in histochemical investigations allows the symptoms of metal intoxication in animal cells to be assessed.

The objective of the current study was to assess the enzyme marking the activity of the endoplasmic reticulum involved in the production of proteins in the cells of the submandibular salivary gland of rats exposed to cadmium and/or zinc in their drinking water.

Material and Methods

The study, which was approved by the local Ethics Committee for Experiments on Animals, used 144 male Wistar rats, aged 5–6 weeks, with an initial weight of 180–200 g. Throughout the experiment, the animals were kept in the same environmental conditions and were fed a wholesome granulated linseed meal (LSM) diet.

The animals had unlimited access to food and drinking water. The control animals were given cadmium- and zinc-free water to drink, whereas the rats in the study groups, i.e. those exposed to the metals, received aqueous solutions of cadmium chloride ($CdCl_2$) at doses of 5 mg Cd/dm^3 or 50 mg Cd/dm^3. Zinc was given in the form of zinc chloride ($ZnCl_2$) at concentrations of 30 mg Zn/dm^3 or 60 mg Zn/dm^3.

The level of cadmium in the blood and urine of the experimental animals exposed to 5 mg Cd/dm^3 were comparable to the concentrations observed in humans at environmental exposure, especially in smokers. The exposure of rats to 50 mgCd/dm^3 corresponds to occupational exposure in humans.

The experiment was conducted in two series with varying lengths of exposure to the metals. Series 1 consisted of 9 groups: one control and eight groups exposed to cadmium and/or zinc for 6 months (72 rats). Series 2 involved analogous groups exposed to the metals for 12 months (72 rats).

In each series, the division of the animals into nine groups (8 rats in each) was based on the type and dose of the metals:

– The control group: the animals were given cadmium-free and zinc-free water to drink,

– Group Zn30: the animals were given zinc at a concentration of 30 mg/dm^3,

– Group Zn60: the animals received zinc at a concentration of 60 mg/dm^3,

– Group Cd5: the animals received cadmium at a concentration of 5 mg/dm^3,

– Group Cd5 + Zn30: the animals were given both cadmium and zinc (5 mg Cd/dm^3 and 30 mg Zn/dm^3),

– Group Cd5 + Zn60: the animals were given both cadmium and zinc (5 mg Cd/dm^3 and 60 mg Zn/dm^3),

– Group Cd50: the rats were given cadmium at a concentration of 50 mg/dm^3,

– Group Cd50 + Zn30: the rats received both cadmium and zinc (50 mg Cd/dm^3 and 30 mg Zn/dm^3),

– Group Cd 50 + Zn60: the animals were given both cadmium and zinc (50 mg Cd/dm^3 and 60 mg Zn/dm^3).

Male Wistar rats were used for the experiment due to their metabolism being similar to that of humans. This is the reason this breed of rats is commonly used to assess the effects of heavy metals on the human body.

After exposure was terminated, the animals were killed under deep anesthesia induced by administering Vetbutal (Biovet, Poland), following the recommendations of the local Ethics Committee for Experiments on Animals.

Submandibular salivary gland tissue was collected and immediately frozen with compressed carbon dioxide. It was cut at –16°C into series of 7 μm sections that were glued to gelated slides. These specimens were used to analyze reactions to glukose-6-phosphatase (G-6-P-ase) (E.C. 3.1.3.9) according to the Wachstein-Meisel method [23] using glucose-6-phosphate sodium salt (G-6-P) (Sigma). The incubation time at 37°C was 26 minutes.

Results

The Control Group

A positive moderate granular-diffuse reaction was localized in the cytoplasm of the serous acinar cells, as compared to the mucous tubules, which showed a weak granular reaction (Fig. 1). A positive reaction of a similar character was observed in the basal and apical parts of the cytoplasm of the cells lining the respective generations of the excretory ducts, from the intercalated ducts to the main duct.

The Zn30 and Zn60 Groups

A slight but noticeable systematic increase was noted in the quantity of the granular-diffuse reaction products r moderate diffuse reaction in the

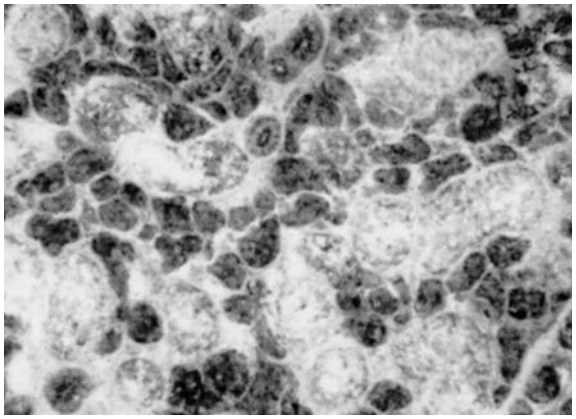

Fig. 1. Reaction to G-6-P. Light microscope. Notch of submandibular gland, control group, series I (magn. 240×)

Ryc. 1. Reakcja na G-6-P, mikroskop świetlny, wycinek ślinianki podżuchwowej szczura grupy kontrolnej serii I, pow. 240×

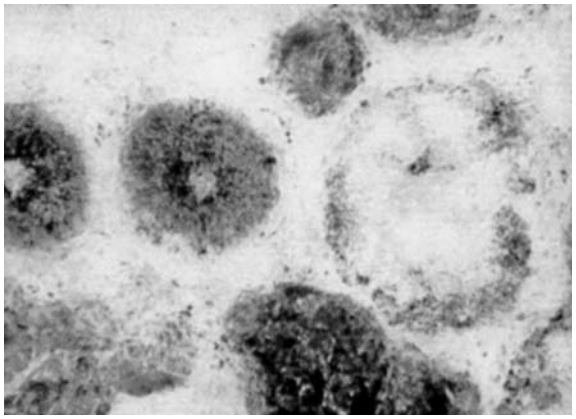

Fig. 2. Reaction to G-6-P, light microscope, notch of submandibular gland, Zn60 group, series I (magn. ×800)

Ryc. 2. Reakcja na G-6-P. Mikroskop świetlny. Wycinek ślinianki podżuchwowej szczura grupy Zn60 serii I, pow. 800×

cytoplasm of both the serous cells and of the cells lining the intralobular excretory ducts (Fig. 2). In the cells of the mucous tubules and intralobular ducts, the reaction to the enzyme examined remained at the level of the control group.

The Cd5 Group

No differences in the intensity, nature and location of G-6-P-ase in the secretory and excretory segments of the submandibular salivary gland were noted in this group as compared to the control animals.

The Cd50 Group

In some of the serous lobules and acini, the cytoplasm showed a noticeable change in the character of the enzymatic reaction product: Instead of the moderately granular-diffuse reaction observed in the control animals, the reaction in this group was moderately diffuse – granular (Fig. 3). No differences in the character, intensity and location of the reaction product in comparison to the control group were found in either the intralobular or interlobular excretory ducts.

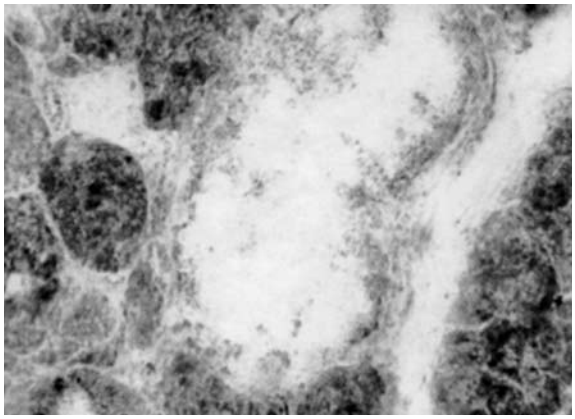

Fig. 3. Reaction to G-6-P, light microscope, notch of submandibular gland, Cd50 group, series I (magn. ×800)

Ryc. 3. Reakcja na G-6-P, mikroskop świetlny, wycinek ślinianki podżuchwowej szczura grupy Cd50 serii I, pow. 800×

The Cd5 + Zn30 Group

The reaction to G-6-P-ase in this group did not differ in character, intensity or distribution of the reaction product from the reaction observed in the control group.

The Cd5 + Zn60, Cd50 + Zn30 and Cd50 + Zn60 Groups

A slight activation of the reaction to G-6-P-ase was noted in all three of these groups, both in the serous and mucous part of the gland and in the intralobular excretory ducts, especially in the mucous tubules (Figs. 4 and 5) as compared to the control animals. In the majority of the serous acini, the reaction had either a diffuse-macrogranular nature or the character of a reaction product grain. Stained grains containing G-6-P-ase, relatively irregularly distributed in the cytoplasm of the serous cells, formed large or very large conglomerates.

Discussion

The enzyme glucose-6-phosphatase (G-6-P-ase) chosen for the current experiment and other in-

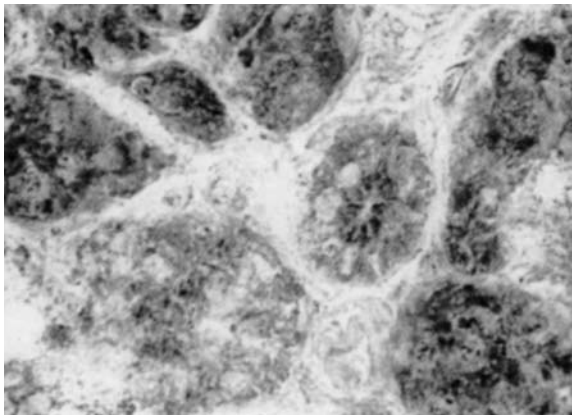

Fig. 4. Reaction to G-6-P, light microscope, notch of submandibular gland, Cd5+Zn60 group, series I (magn. ×800)

Ryc. 4. Wycinek ślinianki podżuchwowej szczura grupy Cd5+Zn60 serii I, reakcja na G-6-P, pow. 800×

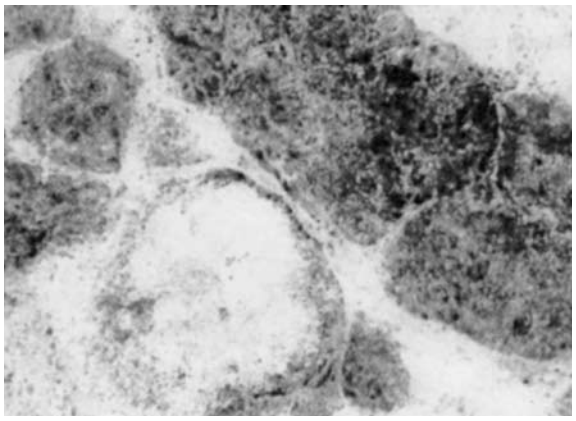

Fig. 5. Reaction to G-6-P, light microscope, notch of submandibular gland, Cd50+Zn60 group, series I (magn. ×800)

Ryc. 5. Wycinek ślinianki podżuchwowej szczura grupy Cd50+Zn60 serii I, reakcja na G-6-P, pow. 800×

dicatory enzymes known from literature have frequently been applied by researchers to assess the reaction of organs and tissues to the toxic action of xenobiotics [24, 25]

The G-6-P-ase marking the endoplasmic reticulum in the cells only in the submandibular glands of animals exposed to environmental and occupational doses of zinc for 6 months showed an increase in the amount of normal enzymatic reaction products. In the groups receiving cadmium or both zinc and cadmium at both doses, this increase was accompanied by either a change in the narture of the reaction, with the diffuse component predominating, or a tendency to form large conglomerates of the product grains. All of these alterations in the intensity and character of the reaction to G-6-P-ase were observed at the secretory level of the salivary gland and only in the serous acini. The organ-related regionalism of G-6-P-ase activity may indicate a special role for serous cells in metal deposition, and perhaps in metallothionein production.

In the animals that underwent 12 months of exposure to the metals, both doses of zinc were still found to increase the amount of the diffuse-granular G-6-P-ase reaction product only in the serous part of the gland. In the groups exposed to cadmium and to cadmium and zinc jointly for 12 months, instead of this increase there was a definite reduction in the amount of reaction product in the serous and mucous parts of the organ. These findings seem to indicate that in the group of animals exposed to the metals for 12 months, damage to the endoplasmic reticulum was not regional, but also involved the serous and mucous parts. These results are also confirmed by ultrustructural investigations [26] that revealed cytoplasmic regions in the glandular cells with marked degranulation of the rough endoplasmic reticulum that underlies protein synthesis, potentially including metallothionein. Damage to the endoplasmic reticulum may be associated with hindered synthesis of metallothionein or other proteins, impaired metal deposition in the salivary gland or a disorder that intensifies the damage. Thus, a fundamental role in the response of the salivary gland to cadmium and/or zinc can be ascribed not only to the ATP--generating mitochondria that regulate calcium metabolism and the outflow of reactive oxygen species from the matrix, but also the endoplasmic reticulum synthesizing various types of proteins. The results of the histochemical investigation seem to indicate that the submandibular salivary gland can synthesize MT, especially within the serous cells. Apart from the higher enzymatic and structural sensitivity to the metals confirmed by the current experiment, the serous cells possess excellent structural conditions to carry out the synthesis: numerous mitochondria, well-developed endoplasmic reticula, Golgi apparatuses and extensive vacuolar apparatuses. This is also confirmed by the findings reported by Zalewska, who emphasizes that the submandibular salivary gland deposits cadmium and actively participates in its systemic turnover [27]. The author suggests that the main factor in this role of the salivary gland is its perfect vascular supply.

The results and hypotheses described above confirm the findings of an earlier study on metallothionein levels in the salivary gland using the same experimental model [28]. After a 6-month exposure to cadmium, animals receiving 5 mg Cd/ dm^3 had a higher level of metallothionein (MT) than the control group. In a group exposed to

50 mg Cd/dm^3, there was a further increase in the level of MT in the rat submandibular salivary gland.

In the current study, after a 12-month-exposure to Cd, the level of MT in the salivary gland was higher in the two experimental groups than in the control group, which coincides with the activity level of the endoplasmic reticulum of the cells of the submandibular salivary gland. An earlier study also confirmed that the accumulation of cadmium and zinc in the submandibular salivary gland activates MT synthesis in the organ [29].

MT-I and MT-II expression was found in the salivary gland by Sunardhi-Widyaputra [30], while Irie [31] confirmed *in vitro* the biosynthesis of metallothionein III (MT-III) in the human salivary gland. MT-III is structurally higher than MT-I and MT-II, because it contains 68 amino acids. Cadmium has been found to induce MT in the liver and testes of rats, but the different mRNA levels accompanying the formation of MT in those organs may suggest that different forms of MT are synthesized, in different ways. In the present authors' earlier investigations, cadmium deposition in the parenchyma of the submandibular salivary gland was found to increase with elevated levels of this metal and longer exposure time. The observed increase, although statistically significant, was by far lower than the elevation of Cd levels in the target organs for this metal, i.e. in the liver and kidneys. The increase noted in the activity of G-6-P-ase and the level of metallothionein in the submandibular salivary gland during a 6-month period of exposure to Cd may suggest MT involvement in Cd deposition in this gland. It is difficult to propose any definite explanation of the reduction observed in the MT level in the salivary gland and G-6-P-ase activity after a 12-month-exposure to cadmium. It seems that MT is mainly involved in the initial period of exposure to this metal. Prolonging the period of exposure to Cd may trigger mechanisms that excrete cadmium with the saliva as one of the major ways of eliminating it from the body.

To sum up, it should be noted that the activity of glucose-6-phosphatase changed in the cells of the rat submandibular salivary gland in response to the toxic action of cadmium and zinc, especially when higher doses were used. The results obtained for the action of the enzyme marking the activity of the endoplasmic reticulum are consistent with the ultrastructural picture and evaluation of MT level in the tissue of the submandibular salivary gland of rats exposed to cadmium and/or zinc in their drinking water in the experimental model presented. Thus, it can be stated that the submandibular salivary gland, along with the liver and kidneys, is a major organ involved in the metabolism of cadmium and zinc.

References

[1] **Cousins RJ:** Regulatory aspects of zinc metabolism in liver and intestine. Nutr Rev 1979, 97, 37–42.

[2] **Dąbrowska E, Szmitkowski M, Kulikowska-Karpińska E, Łapińska J, Letko R:** Cadmium content in the rat submandibular salivary gland depending on Cd dose and exposure time. Pol J Environ Stud 2009, 18, 6A, 169–172.

[3] **Urbanek-Karłowska B, Wojciechowska-Mazurek M, Starska K:** Ocena zawartości kadmu w wybranych grupach produktów spożywczych. Med Pr 1995, 46, 5, 57–69.

[4] **Benedetti JL, Dewailly E, Turcotte F, Lefabvre M:** Unusually high blood cadmium associated with cigarette smoking among three subgroups of the general population. Sci Total Environ 1994, 152, 161–167.

[5] **Reeves PG, Rossow KL:** Zinc-and/or cadmium-induced intestinal metallothionein and copper metabolism in adult rats. Nutr Biochem 1996, 7, 128–134.

[6] **Mukhopadhay B, Bose S, Bhattacharyya S:** Induction of metallothionein in rat liver by cadmium chloride: probable mechanism of action. Biomed Environ Sci 1994, 7, 232–240.

[7] **Hałatek T, Chmielnicka J:** Ocena nefrotoksycznego działania kadmu u zwierząt doświadczalnych i ludzi. Post Hig Med Dośw 1993, 47, 5, 375–391.

[8] **Cherian MG, Nordberg M:** Cellular adaptation in metal toxicology and metallothionein. Toxicology 1983, 28, 1–15.

[9] **Vasak M, Hasler D:** Metallothioneins: new functional and structural insights. Curr Opin Chem Biol 2000, 4, 177–183.

[10] **Friedman PA, Gesek FA:** Cadmium uptake by kidney distal convoluted tubule cells. Toxicol Appl Pharmacol 1994, 128, 2, 257–263.

[11] **Brzóska MM, Kamiński M, Dziki M, Moniuszko-Jakoniuk J:** Changes in the structure and function of the kidney of rats chronically exposed to cadmium. II Histoenzymatic Studies. Arch Toxicol 2004, 78, 4, 226–231.

[12] **Orłowski C, Piotrowski JK, Bem EM, Szumiło J:** The monitoring of cadmium, zinc and copper in the kidneys and liver of deceased humans from the region of Lublin. Acta Pol Toxicol 1995, 3, 99–106.

[13] **Stohs J, Bagachi D:** Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med 1995, 18, 321–336.

[14] **Żak I, Steibert E:** Biochemiczne aspekty toksykologii kadmu. Post Hig Med Dośw 1980, 34, 249–272.

[15] **Pasternak K, Papierkowski A, Majdanik M:** Wpływ niektórych metali na stężenie magnezu i wapnia w mitochondriach wybranych tkanek szczura. Ann Univ Mariae Curie-Skłodowska Lublin – Polonia 2001, 14, 8, 55–60.

[16] **Norbert GF, Kjellstrom T, Norberg M:** Kinetics and metabolism. Cadmium and health: a toxicological and epidemiological appraisal. Eds.: Friberg L, Elinder CG, Kjellstrom T, Norberger G, inc., Boca Raton, Florida 1985, 1, 103–178.
[17] **Martel J, Marion M, Denizeau F:** Effect of cadmium on membrane potential in isolated rat hepatocytes. Toxicology 1990, 60, 161–172.
[18] **Skoczyńska A, Smolik R:** The effect of combined exposure to lead and cadmium on serum lipids and lipid peroxides level in rats. Int J Occup Med Environ Health 1994, 7, 263–271.
[19] **Szpetnar M, Pasternak K:** Aktywność dysmutazy ponadtlenkowej w tkankach szczurów otrzymujących chlorek kadmu. Ann Univ Mariae Curie – Skłodowska Lublin – Polonia 2001, 14, 6, 45–48.
[20] **Putman CD, Arvai AS, Bourne Y, Tainer JA:** Active and inhibited human catalase structures: ligand and NADPH binding and catalytic mechanism. J Mol Biol 2000, 296, 295–309.
[21] **Kulikowska E, Moniuszko-Jakoniuk J, Miniuj K:** Rola cynku w procesach fizjologicznych i patologicznych organizmu. Pol Tyg Lek 1991, 46, 24–26, 470–473.
[22] **Kojima S, Furukawa A, Kiyozumi M, Shimada H:** Comparative renal toxicity of metallothioneins with different cadmium/zinc ratios in rats. Toxicol Lett 1991, 56, 197–205.
[23] **Wachstein M, Meisel E:** Histochemistry of hepatic phosphatases and physiologic pH with special reference to the demonstration of bile canaliculi. Ann J Clinic Pathol 1957, 27, 12–23.
[24] **Dąbrowska E, Balunowska M, Szynaka B, Letko R:** Biological effect of natrium fluoride in chosen internal organs of the rat – ultrastructural study. Acta Toxicol 2005, 13, 1, 31–36.
[25] **Kulkarni JS, Khanna A:** Functional hepatocyte-like cells derived from mouse embryonic stem cells: A novel in vitro hepatotoxicity model for drug screening. Toxicol *in vitro* 2006, 20, 6, 1014–1022.
[26] **Dąbrowska E, Szynaka B, Kulikowska-Karpińska E:** Ultrastructural study of the submandibular gland of the rat after 6-month exposure to cadmium and zinc in drinking water. Adv Med Sci 2006, 51, 245–249.
[27] **Zalewska A, Brzóska MM, Marciniak J, Karaszewska K, Zwierz K, Moniuszko-Jakoniuk J:** Activity of lysosomal exoglycosidases in submandibular glands of rats intoxicated by cadmium at doses related to human chronic environmental and occupational exposures. Acta Biochim Pol 2004, 51, 3, 831–837.
[28] **Dąbrowska E, Letko R, Gałażyn-Sidorczuk M, Kulikowska-Karpińska E, Łapińska J:** Effect of exposure to cadmium on the level of metallothionein in the rat submandibular gland. Pol J Environ Stud 2009, 18, 6A, 186–188.
[29] **Dąbrowska E, Szmitkowski M, Kulikowska-Karpińska E, Łapińska J, Letko R:** Effect of zinc supplementation on cadmium concentration in the rat submandibular salivary gland. Pol J Environ Stud 2009, 18, 6A, 173–181.
[30] **Sunardhi-Widyaputra S, Van den Oord JJ, Van Houdt K, De Ley M, Van Damme B:** Identification of metallothionein- and parathyroid hormone-related peptide (PTHrP)-positive cells in salivary gland tumors. Path Res Pr 1995, 191, 11, 1092–1098.
[31] **Irie Y, Mori F, Keung WM, Mizushima Y, Wakabayashi K:** Expression of neuronal growth inhibitory factor (metallothionein-III) in the salivary gland. Phys Res 2004, 53, 6, 719–723.

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Conflict of interest: None declared

Received: 11.12.2010
Revised: 27.01.2011
Accepted: 2.06.2011