Impact of cadmium on the level of hepatic metallothioneins, essential elements, and selected enzymes in the experimental rat model

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ABSTRACT: The response of different strains of laboratory rats (*Rattus norvegicus* L.) on both acute (*via* intraperitoneal injection) and chronic (*via* drinking water and/or diet) cadmium intoxication was investigated in the model study. The rat strains Long Evans (LE), Spontaneously hypertensive rat (SHR), and Brown Norway (BN) were tested and compared, and total Cd levels and metallothionein (MT) concentrations were determined in the liver of experimental animals. The liver MT concentrations were determined by using adsorptive chronopotentiometry and modified Brdička reaction and were significantly correlated (r = 0.965) with the total liver Cd content. Moreover, the Cd application resulted in increasing zinc liver contents confirming intensive MT synthesis in the rat liver. In the blood plasma, specific enzymatic activity of glutathione reductase (GR) and glutathione-*S*-transferase (GST) was determined suggesting increasing activity of GR with the amount of applied Cd for all three strains, whereas ambiguous results have been found for the activity of GST. Therefore, MT concentrations seemed to be more sensitive indicators of the Cd intoxication compared to the assessment of the specific enzymatic activity.

Keywords: metal intoxication; liver; blood plasma; detoxification; *Rattus norvegicus* L.

INTRODUCTION

The distribution of cadmium among organs of animals differs depending on the chemical form of administered Cd and the duration of exposure. Long-term exposure to high doses of Cd may cause biochemical and functional changes in some critical organs. Moreover, Cd can influence the absorption and distribution of essential elements and can replace them in enzymes (Swiergosz-Kowalewska 2001). Metallothionein (MT) is a common name for a huge group of low molecular weight proteins (6–7 kDa) capable of binding d^{10} metal ions through the 20 cysteinyl groups that constitute part of the structure. The most widely expressed isoforms in mammals are MT-1 and MT-2, where 60–61 amino acids form structure with two clusters, able to bind seven metal ions: $Me^{II}_3S_9$ and $Me^{II}_4S_{11}$. The naturally existing MT form only bounds zinc. With the exposition of organism to Cd, synthesis of MT is induced and Cd replaces zinc to form structure Cd_5Zn_2MT (Klaassen et al.

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1999, Vasak 2005). MT plays important roles in Cd retention in tissues and dramatically decreases biliary excretion of Cd. Cd-bound to MT is responsible for Cd accumulation in tissues and the long biological half-life of Cd in the body. Induction of MT protects against acute Cd-induced lethality, as well as acute toxicity to the liver and lung (Klaassen et al. 2009).

A common model used in toxicology to estimate the potential hazard of contaminants for human health is rat, where after exposition to Cd, induction of MT in several tissues, especially in liver, has been well documented (Chan et al. 1992a, Szakova et al. 2009). Comparing to liver, Cd induces MT to a lesser extent in the testes indicating the higher susceptibility of testis to Cd toxicity. Moreover, the significant reduction of daily sperm production in intraperitoneally treated rats exposed to high Cd dose was accompanied by the decrease of MT level (Xu et al. 2005). Different response of Sprague-Dawley rats on Cd and Hg intraperitoneal exposition was observed by Chan et al. (1992a). The presence of Hg induced the synthesis of MT only in kidney but not in liver, whereas Cd injections induced MT synthesis in both liver and kidney. Even exogenously administered MT to rats showed protective effect against potential liver damage (Kara et al. 2005). The induction of MT could be affected by co-exposure to more elements. Wang et al. (2009) exposed the Sprague-Dawley rats to Cd and Pb in drinking water for 8 weeks to investigate the possible combined effect of these metals on the expression of MT in kidney. They found significant increase of the MT expression during the combined exposition to the elements indicating their possible interaction in the rat organism. In the opposite, zinc can protect against acute CdMT nephrotoxicity of mice (Tang et al. 1998) and testes damage in Wistar rats (Amara et al. 2008). Selenium application can increase the protective effect of Zn against the Cd toxicity. Jihen et al. (2009) documented that Se or Zn administration during exposure to Cd has only partial corrective effects on Cd-induced oxidative stress in liver while Se and Zn together assured a more efficient protection of the organ against the observed oxidative stress in male Wistar rats.

Several methods of MT detection and quantification including separation techniques, electrochemical methods, immunological methods, and quantification of MT mRNA were developed and comprehensive reviews have been published lately. Among them, differential pulse voltammetry (DPV), chronopotentiometric stripping analysis (CPSA), and high-performance liquid chromatography (HPLC) or capillary electrophoresis (CE) coupled with inductively coupled plasma mass spectrometry (ICP-MS) are characterized by the lowest limits of detection (Dabrio et al. 2002; Adam et al. 2010). Among the electrochemical techniques, the application of constant current chronopotentiometry gives better separation of signals than voltammetry (Sestakova and Navratil 2005; Serrano et al. 2006). For separation of the individual MT isomers, CE belongs to the most preferred techniques (Beattie 1998). However, a competitive enzyme-linked immunosorbent assay (ELISA) for the determination of MT in tissues and body fluids has been developed and verified with an acceptable limit of detection (Chan et al. 1992b).

In this study, Spontaneously hypertensive rats (SHR) (Szpirer et al. 1996) and Brown Norway (BN) (Pravenec et al. 1996, 1997; Pravenec and Kurtz 2010) were chosen as genetically far distant models. For comparison, outbred strain Long Evans (LE) with less than 1% relation in generation was used. This represents the most common model for animal research (Pravenec and Kurtz 2010). Moreover, the relationships between Cd exposition, synthesis of MT, and activity of two important enzymes – glutathione reductase (GR) and a group of glutathione *S*-transferases (GSTs) at different rat strains were followed. Glutathione (GS) and GR are important for many cellular functions, cell cycle regulation, damage reparation, and metabolism and detoxification of xenobiotics (Faut et al. 2013). GSTs are a group of enzymes with important function in organism detoxification. GST catalyzes reaction between reduced glutathione (GSH) and a wide range of electrophile substances: RX + GSH → HX + RGS. *Via* this mechanism, GSTs participate in detoxification of xenobiotics (Board 1981). Jadhav et al. (2007) had exposed male Wistar rats to the element mixture (arsenic, cadmium, lead, mercury, chromium, nickel, manganese, iron) in drinking water for 90 days. The results demonstrated that the subchronic exposure of male rats to the mixture of metals *via* drinking water results in induction of oxidative stress and concomitant reduction in antioxidative defense system in erythrocytes. According to the results of the mentioned studies the following hypothesis was set up: the Cd uptake and accumulation as well

as MT synthesis and the activity of detoxification enzymes in the rat organism are strongly affected by the individual rat strains. The main objective of the study was to assess the effect of the individual rat models on the interpretation of the results of various indicators characterizing response of the rat organism exposed to Cd.

MATERIAL AND METHODS

Experimental design. 90-day-old male rats of Long Evans strain (LE), Spontaneously hypertensive rats (SHR), and Brown Norway rats (BN) were fed standard diet ST-1 Bergman (14% moisture, 21% nitrogen, 3% fat, 4.1% pulp, 1% Ca, 0.8% P, 0.2% Mg, and 0.25% Na). All animals were housed in cages (1 animal per cage) in a room with controlled temperature (23–25°C) under natural light conditions. Cadmium as $CdCl_2$ was applied as follows: for an assessment of long-term effect of Cd this compound was (*i*) dissolved in drinking water and applied for 60 days in concentration of 10 mg of Cd as $CdCl_2$ per 1 l of water, (*ii*) mixed to the diet in the amount of 10 mg of Cd as $CdCl_2$ per 1 kg of feeding mixture for 60 days. For an assessment of a single dose of Cd application Cd was applied as 0.027 mol/l $CdCl_2$ solution by the injection into peritoneum in amounts corresponding to 0.3 mg or 0.6 mg of Cd per 100 g of body weight for MT and element determination and 1.25 mg and 2.5 mg per 100 g of body weight for enzyme assay, always twice – 72 and 24 h before sample collection. After the termination of the experiment the animals were euthanized by exsanguination after anaesthetizing with Xylapan (xylasin) and Narketan (ketamin) and whole blood and liver were sampled. The sampled tissues were kept at –18°C for MT determination, aliquots of them were freeze-dried and homogenized for element determination; aliquots of blood samples were taken into heparinized tubes. Blood plasma was obtained by centrifugation at 2500 *g* at 4°C for 10 min.

Analytical methods. For determination of total content of elements in liver, aliquots (0.5 g) of freeze-dried and homogenized liver samples were weighed into a digestion vessel. Concentrated nitric acid (8.0 ml) (Analytika Ltd., Prague, Czech Republic), and 30% H_2O_2 (2.0 ml) (Analytika Ltd.) were added. The mixture was heated in an Ethos 1 (MLS GmbH, Leutkirch, Germany) microwave assisted wet digestion system at 220°C for 30 min. After cooling, the digest was quantitatively transferred into a 20-ml glass tube and filled up to the volume by deionized water. A certified reference material MB 12-02-01 Bovine liver (Analytika Ltd.) was applied for the quality assurance of analytical data. In this material the content of 0.48 ± 0.03 mg/kg Cd, 0.325 ± 0.014 mg/kg Se, and 162 ± 6 mg/kg Zn is certified. In our experiment, 0.54 mg/kg Cd, 0.290 mg/kg Se, and 163 mg/kg Zn was determined. Total contents of Cd and Zn in the digests were determined by optical emission spectroscopy with inductively coupled plasma (ICP-OES) with axial plasma configuration (VistaPro; Varian Australia Pty Ltd., Mulgrave, Australia), equipped with autosampler SPS-5. Measurement conditions for all lines were: power 1.2 kW, plasma flow 15.0 l/min, auxillary flow 0.75 l/min, nebulizer flow 0.9 l/min. For determination of low concentrations of Cd in the digests, electrothermal atomic absorption spectrometry (ETAAS) using the instrument Varian AA280Z (Varian Australia Pty Ltd.) equipped with a GTA120 graphite tube atomizer was applied. The total selenium concentrations in the digests were determined by hydride generation atomic absorption spectrometry using the instrument Varian AA280Z equipped with a continuous hydride generator VGA-77.

For MT determination, 200 mg of liver were homogenized at 0°C within 2 ml of Tris buffer (pH 8.2), then an aliquot of 500 µl was diluted by 0.1 mol/l NaCl in the ratio 1 : 9 and heated at 95°C for 5 min. Supernatant obtained after centrifugation (30 000 *g*, 4°C, 20 min) containing heat-stable MT was used for MT determination. The electrochemical method based on modified Brdička reaction (Olafson 1991; Raspor 2001; Erk et al. 2002) was used. Chronopotentiometric measurement was performed using the PC-ETP voltammetric analyzer (Polaro-Sensors, Prague, Czech Republic), equipped with POLAR.PRO software (Version 5.1). Pen-type hanging mercury drop electrode was used as the working electrode, $Ag/AgCl/KCl_{sat}$ as a reference electrode, and platinum wire served as a counter electrode (both Elektrochemické detektory, Ltd., Turnov, Czech Republic). The pH was measured by a digital pH/mV meter MPH 61 with combined electrode TYPE 01-29 (all MONOKRYSTALY, s.r.o., Turnov, Czech Republic). A new drop was used for each record; measurement was performed in nitrogen atmosphere. For quantification of MT concentration, standard addition mode was used. For MT deter-

mination, rabbit MT (Cd-Zn-MT, lot 052K7012; Sigma-Aldrich Corp., St. Louis, USA) was used. All chemicals were of analytical purity grade, deionized water (0.054 μS) (Milli-Q-Gradient; Millipore, Billerica, USA) was used.

Determination of specific enzymatic activity of glutathione reductase was performed by using the following procedure: oxidized glutathione (GSSG) was reduced by glutathione reductase and NADPH to reduce glutathione (GSH). GSH than spontaneously reacted with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) forming 5-thio(2-nitrobenzoic acid) (TNB) where the TNB concentration was measured at λ = 412 nm. Reaction mixture was prepared by mixing 50 μl of reaction buffer (100 mmol/l phosphate buffer (pH 7.5), 1 mmol/l EDTA, 250 μl of 2 mmol/l GSSG dissolved in the reaction buffer, 125 μl 3 mmol/l DTNB (all Sigma-Aldrich Corp.) dissolved in the reaction buffer, and 50 μl of the plasma sample. The reaction started after addition of 25 μl of 2 mmol/l NADPH. The reaction was performed in 1 cm quartz cuvette at 25°C and absorbance was measured continuously for 4 min (Glatzle et al. 1974).

Determination of specific enzymatic activity of glutathione *S*-transferase was performed as follows: GSH reacted under glutathione *S*-transferase catalysis, with 1-chlor-2,4-dinitrobenzene (CDNB) forming GS-DNB conjugate and HCl where the GS-DNB concentration was measured at λ = 340 nm. The reaction mixture was prepared by mixing 210 μl of reaction buffer (100 mmol/l phosphate buffer (pH 6.5), 0.1% v/v Triton X-100; Sigma-Aldrich Corp.), 250 μl of 2 mmol/l GSH (Sigma-Aldrich Corp.) dissolved in reaction buffer, and 25 μl of plasma sample. The reaction started with pipetting 15 μl of 33.3 mmol/l CDNB (Fluka Chemie GmbH, Buchs, Switzerland) dissolved in ethanol into reaction mixture. The reaction was performed in 1 cm quartz cuvette at 25°C and absorbance was measured continuously for 4 min (Habig et al. 1974).

Statistical methods. The data obtained for Cd concentrations in liver were subjected to Dixon's test for identification of outliers (significance level α = 0.05) using MS Excel 2007. Subsequently, one-way analysis of variance was used at the significance level α = 0.05 (STATISTICA software package, Version 9.1, 2011). Correlation analysis was used for assessment of the relationships among the variables where Pearson's correlation coefficients were applied for the data characterized by the normal data distribution and non-parametric Spearman's correlation was used in the remaining cases (Meloun and Militky 2004).

RESULTS

The Cd contents in rat liver were comparable for all three different rat strains, with the exception

Table 1. Elements content (mg/kg DM) in the liver of experimental rats (n = 6)

Strain	Treatment	Cd	Se	Zn
LE	control	0.266 ± 0.157^{a}	0.320 ± 0.068^{a}	78.9 ± 7.0^{a}
	Cd in diet (10 mg/kg)	11.1 ± 2.6^{b}	0.184 ± 0.108^{a}	97.0 ± 22.7^{a}
	injected 0.3 mg Cd/100 g BW	74.6 ± 8.1^{c}	0.232 ± 0.148^{a}	144 ± 21^{b}
SHR	control	0.372 ± 0.135^{a}	0.107 ± 0.058^{a}	88.0 ± 4.0^{a}
	Cd in water (10 mg/l)	8.88 ± 0.90^{c}	0.060 ± 0.044^{a}	92.4 ± 9.5^{a}
	Cd in diet (10 mg/kg)	1.70 ± 0.09^{b}	0.104 ± 0.035^{a}	71.7 ± 10.1^{a}
	injected 0.3 mg Cd/100 g BW	98.2 ± 16.6^{d}	0.062 ± 0.026^{a}	156 ± 15^{b}
	injected 0.6 mg Cd/100 g BW	188 ± 34^{e}	0.078 ± 0.051^{a}	122 ± 27^{b}
BN	control	0.614 ± 0.413^{a}	0.056 ± 0.022^{a}	99.8 ± 3.5^{a}
	Cd in water (10 mg/l)	6.25 ± 1.12^{b}	0.038 ± 0.011^{a}	120 ± 12^{bc}
	injected 0.3 mg Cd/100 g BW	102 ± 32^{c}	0.064 ± 0.022^{a}	151 ± 13^{c}
	injected 0.6 mg Cd/100 g BW	155 ± 56^{c}	0.050 ± 0.043^{a}	152 ± 23^{c}

BW = body weight, DM = dry matter, LE = Long Evans, SHR = Spontaneously hypertensive rat, BN = Brown Norway
$^{a–d}$averages marked by the same letter did not significantly differ at $P < 0.05$ within individual columns and strains
data are presented as mean ± standard deviation

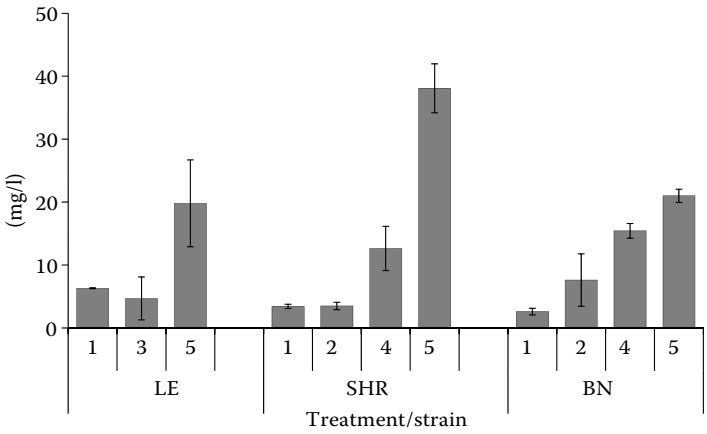

Figure 1. Average contents of metallothioneins in the liver extracts (mg/l) of rats (n = 6)

treatments: 1 = control, 2 = Cd in diet (10 mg/kg), 3 = Cd in water (10 mg/l), 4 = injected 0.3 mg Cd per 100 g of body weight, 5 = injected 0.6 mg Cd per 100 g of body weight

data are presented as mean ± standard deviation

of intraperitoneal acute intoxication with 0.6 mg Cd per 100 g of body weight, where the highest value was found for SHR strain (Table 1). The critical concentration of Cd in the liver tissue of small rodents is 20–30 mg/kg (Swiergosz-Kowalewska 2001). Therefore, the Cd contents in the liver after the acute intraperitoneal intoxication exceeded this value whereas the oral exposition of the animals resulted in lower final Cd contents. Among the elements discussed, regarding their potential interaction with the increasing Cd content in animal tissues, Se and Zn were determined (Table 1). Whereas Se contents in liver were low and did not differ among individual strains and/or Cd application mode, the Zn contents in liver increased significantly ($P < 0.05$) with increasing Cd content. The Zn contents were significantly ($P < 0.05$) related to the Cd contents where y(Zn) = 0.34x(Cd) + 95.4, and r = 0.559 confirming the results summarized by Swiergosz-Kowalewska (2001).

Figure 1 summarizes the MT concentrations in the liver extracts where no significant differences occurred between LE and BN strains whereas higher MT concentrations were determined in SHR strain. For all the strains, the MT values correlated with the liver Cd content where the equation y = 0.194x + 2.86 has the correlation coefficient r = 0.97 which confirms linearity ($P < 0.05$). This is in agreement with data from literature (Hogstrad and Haux 1992; Dallinger et al. 1999), where for dependence of MT content on Cd concentration correlation coefficients 0.96 or 0.86 were reported. A similar range of MT concentrations, when calculated for 1 g of liver (51–569μM), was reported for example in the liver and kidney of fish samples (Krizkova et al. 2010) suggesting the similar rate of the MT synthesis regardless of the animal species.

Table 2. Average contents (U/ml) of detoxification enzymes determined in blood plasma of experimental rats (n = 6)

Treatment	Strain		
	BN	SHR	LE
Glutathione reductase (U/ml)			
Control	0.014 ± 0.002[a]	0.008 ± 0.002[a]	0.007 ± 0.002[a]
Cd in water (10 mg/l)	0.017 ± 0.002[a]	0.007 ± 0.001[a]	0.008 ± 0.002[a]
Injected 0.6 mg/100 g LV	0.018 ± 0.002[b]	0.018 ± 0.003[b]	0.017 ± 0.002[b]
Injected 1.2 mg/100 g LV	0.030 ± 0.004[b]	0.023 ± 0.002[b]	0.014 ± 0.004[b]
Glutathione *S*-transferase (U/ml)			
Control	0.014 ± 0.002[a]	0.014 ± 0.003[a]	0.016 ± 0.003[a]
Cd in water (10 mg/l)	0.017 ± 0.002[a]	0.016 ± 0.004[a]	0.013 ± 0.002[a]
Injected 0.6 mg/100 g LV	0.018 ± 0.002[a]	0.020 ± 0.004[a]	0.036 ± 0.001[b]
Injected 1.2 mg/100 g LV	0.030 ± 0.004[b]	0.033 ± 0.006[b]	0.023 ± 0.012[a,b]

LV = living weight, LE = Long Evans, SHR = Spontaneously hypertensive rat, BN = Brown Norway

[a,b]averages marked by the same letter did not significantly differ at $P < 0.05$ within individual columns

data are presented as mean ± standard deviation

Enzymatic activity of GR in blood plasma of rats (Table 2) exposed to Cd was expected to increase (Ognjanovic et al. 2003; Flora et al. 2008). In our study, there was no significant difference in GR activity between control and application of Cd in water in all three strain groups. A significant ($P < 0.05$) increase was found for both acute intraperitoneal applications. Determination of specific glutathione *S*-transferase activity (GST) showed in the case of LE strain no difference between control group and group with Cd in water. Increase of the specific activity was found in group with intraperitoneal injection of 0.6 mg per 100 g of body weight. At laboratory rat strains SHR and BN, similar relations could be observed. There was again no difference in glutathione *S*-transferase activity between control group and group with Cd in water. We could see a significant ($P < 0.05$) increase of the GST activity in groups with intraperitoneal injection of Cd in the amount of 1.25 mg per 100 g of body weight in both these strains, but there was insignificant increase of glutathione *S*-transferase activity at groups with intraperitoneal injection containing 0.6 mg Cd per 100 g body weight.

DISCUSSION

The risk element uptake and accumulation in animal organism is affected by various factors. Among them, the element application rate, length of the application, age of animal, etc. are the most important. Moreover, the form and/or compound of applied element should be taken into account. Szakova et al. (2009) compared chemical form of Cd applied (as inorganic salt – $CdCl_2$ and organically bound in yeast cells) in diet on its content in the organs of male Wistar rats. The addition of Cd to the diet of rats increased significantly Cd contents in several organs. The addition of yeast containing natural level of Cd increased significantly the content of Cd in the liver and kidney of the experimental animals. There was a significantly increased Cd accumulation in organs after addition of Cd as $CdCl_2$, as compared with the addition of organically bound Cd in yeast cells. Similarly Hispard et al. (2008a) compared administration of inorganic form of Cd ($CdCl_2$ dosed rat food) and organic Cd (contaminated snail-based rat food) in Wistar rats. In their experiment, higher Cd contents were found in kidney and liver of the rats fed organic form of Cd compared to $CdCl_2$ (but without any difference in the case of MT contents) because of high soluble Cd-MT fraction in snails bioavailable to rats. In our case, the significant difference was observed between oral exposure of $CdCl_2$ in water and in the diet where lower bioavailability was observed for the Cd application in the diet, as expectable. However, the results suggested the potential effect of individual rat strains where the SHR rats showed higher ability to Cd accumulation especially in the case of high intraperitoneal acute intoxication with 0.6 mg Cd per 100 g of body weight (Table 1).

In the case of intraperitoneal acute interaction, the results suggested significantly ($P < 0.05$) increased Zn content in liver after intraperitoneal acute intoxication of the animals (Table 1). Swiergosz-Kowalewska (2001) reviewed the increase of Zn levels in liver and kidney of small rodents and its decrease in other tissues as the result of Cd toxicity. This effect was probably caused by an increase in MT synthesis and intensive transportation of Zn from blood plasma to liver and kidney. Also Jihen et al. (2011) observed the increase in Cd accumulation accompanied by an elevation in Zn content in liver. On the other hand, Zn may prevent the effects of Cd because it prevents Cd action on the oxidative phosphorylation in the liver mitochondria as already observed by Friberg et al. (1974). Bernotiene et al. (2012) evaluated the influence of Zn and Se on the antioxidative systems of mice affected by Cd. They applied intraperitoneal injections of $CdCl_2$, $ZnSO_4$, and Na_2SeO_3 solutions to white outbred laboratory mice and observed that Zn and Se prevented liver lipids against Cd-induced peroxidation and did not suppress effects of Cd on MT contents in mice liver. Moreover, the decrease of liver Cd contents after oral application of sodium selenate to rats was observed (Koyuturk et al. 2007). In our case, however, no effect of Cd intoxication of the animals on liver Se contents was observed suggesting negligible effect of Se at low levels of this element.

The MT concentrations in the liver extracts differed according to the Cd application showing lower MT synthesis for chronic Cd application where also the effect of aging should be calculated. For example, Yamano et al. (1999) examined the effects of post-maturational aging on acute Cd-induced hepatotoxicity. The results showed that old (28-month) male Fischer 344 rats were more resistant to liver damage from Cd than younger rats. However, the hepatic MT levels decreased with aging suggesting that the age-associated decrease in sensitivity is not a consequence of enhanced protection from MT. In this context, age-associated changes in Kupffer cell are

important determinants of Cd-induced hepatotoxicity in rats (Yamano et al. 1998). The inter-element interactions related to the Cd-MT induction are affected by individual elements as documented by Saito (2011) where the response of rats to silver injection was investigated and the silver concentrations in the MT fractions of silver-injected rats were higher than those of the control rats although they did not increase in response to injection of silver. Moreover, their results indicated that Zn contents in kidney and Zn concentrations in MT did not increase in response to silver injection. Therefore, the response of MT synthesis to risk elements intoxication of rats is evidently element-specific.

All three strains of laboratory rats used in this study reacted on the injection of Cd, but not on the exposition to Cd in water. Ognjanovic et al. (2003) observed increased activity of antioxidant defense enzymes: copper zinc containing superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione *S*-transferase as a response of male Wistar rats to acute exposure to Cd (0.4 mg Cd/kg of body mass). For glutathione reductase activity, the increase has been observed with the increase of applied Cd concentration for all three strains, in agreement with the literature (Ognjanovic et al. 2003; Flora et al. 2008). In the case of GST activity, a more complex dependence was observed. This could be explained by different function of these enzymes. In GST measurement, strain LE appeared as the most sensitive to Cd exposition – the increase of GST activity was significant already after the intraperitoneal application of 0.6 mg per 100 g body weight.

The increase of the GR and GST activity in blood plasma after Cd injection confirmed that Cd causes induction of oxidative stress and acts toxically on the organisms, which is in agreement with other reports (Neal et al. 1997). The GR activity increased after intraperitoneal application of a small amount of Cd, whereas for GST activity, a higher amount of applied Cd was needed for significant increase in strains SHR and BN. This could be interpreted by a different function of both types of enzymes. GR acts mostly as an antioxidant defense enzyme (Flora et al. 2008). GST acts in detoxification of the organism and so its antioxidative function could be only secondary (Casalino et al. 2006).

Cd applied in water for 60 days did not result in the increase of the investigated antioxidative enzymes. This may be caused by a low absorption of Cd through the digestive tract, because only about 5% is absorbed through this pathway (Jarup et al., 1998). Another reason is a long time of Cd application. In our assay, Cd was applied in a small amount for 60 days. It has been shown that the activity of GR rises up after 30 days of peroral application and after 60 and/or 90 days the activity slightly lowers. That may be connected with other probably non-enzymatic antioxidative mechanisms (Jadhav et al. 2007). Domanska et al. (1999) observed changes in glutathione *S*-transferase activity produced by repeated exposure of male Wistar rats to Cd in the response to each subsequent exposure. It may be suggested that the response of glutathione *S*-transferase depends on the duration of the exposure to Cd, as well. Localization of enzymes may play an important role, too. The *in vitro* experiments showed increased mitochondrial catalase and glutathione reductase (GR) activities more than the cytoplasmic enzymes (Latinwo et al. 2006). Cd is bound to erythrocytes or albumin during blood transport and subsequently is deposited into different tissues. It is possible that the antioxidative mechanisms in blood plasma are not needed after a long-time exposition to a small amount of Cd, blood plasma natural activity may be sufficient for its detoxification.

According to Hispard et al. (2008b), MTS are the most sensitive biomarkers since they are induced in the kidney of rats even at low Cd concentration (2.5 mg Cd/kg) whereas the enzymatic biomarkers are not relevant at such concentration, at least after 4 weeks of exposure. It is in accordance with our results where the MT concentrations reflected significantly the Cd accumulation rate in the liver. Moreover, the results demonstrated differences among the individual rat strains where especially the SHR strain differed from the others showing higher Cd accumulation and subsequently the MT synthesis ability in the liver according to the experimental animals. Thus, the applied experimental rat model can affect the interpretation of the experimental results concerning the assessment and prediction of the risk element behaviour in the animal organism.

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