Polish Journal of Environmental Studies Vol. 14, No 4 (2005), 483-489

Original Research

Involvement of Some Low-Molecular Thiols in the Destructive Mechanism of Cadmium and Ethanol Action on Rat Livers and Kidneys

J. Moniuszko-Jakoniuk*, M. Jurczuk, M. M. Brzóska, J. Rogalska, M. Gałażyn-Sidorczuk

Department of Toxicology, Medical University of Białystok, Mickiewicza 2c, 15-222 Białystok, Poland

Received: October 28, 2004
Accepted: December 19, 2004

Abstract

The involvement of some low-molecular thiol compounds in the mechanisms of peroxidative action of cadmium (Cd) and ethanol (EtOH) was studied. Concentrations of reduced glutathione (GSH), metallothionein (Mt) and thiol (-SH) groups in protein and non-protein fractions were assessed in the homogenates of the liver and kidney of rats exposed to Cd (50 Cd/dm^3 of drinking water) and EtOH (5 g EtOH/kg body weight/24 h, intragastrically), singly or in combination, for 12 weeks.

Exposure to Cd caused a reduction in the concentration of GSH and non-protein SH groups in the liver and kidneys with a simultaneous increase in Mt level in these organs. The concentration of total SH groups increased only in kidneys. Administration of EtOH had no effect on Mt concentration in both organs, but caused a reduction in the concentration of GSH and non-protein SH groups. A reduction in the level of total SH groups following exposure to EtOH was also noted in the liver. In the group of rats with a simultaneous exposure to Cd and EtOH, GSH concentration was decreased in the liver compared to the control and Cd-exposed animals, and in the kidney in comparison to the control and EtOH-receiving rats. Following the combined exposure to Cd and EtOH, the concentration of non-protein SH groups decreased in the liver and kidneys in comparison to the control and Cd-exposed rats, and in the liver also in comparison to the EtOH group. Mt concentration increased in the liver and kidneys of animals exposed to a combination of Cd and EtOH, compared to the control and EtOH group, but was reduced compared to the Cd group. Combined administration of Cd and EtOH caused an increase in the concentration of total SH groups in the kidneys compared to the control, Cd and EtOH groups. A negative correlation was found between GSH concentration and malondialdehyde (MDA) levels and positive correlation between Mt and MDA.

The intensity of lipid peroxidation as well as GSH and Mt concentrations influencing this process in the state of combined exposure to Cd and EtOH results both from independent actions of these substances and interactions between them.

The study outcome seems to indicate that the Cd- and EtOH-induced reduction in GSH and non-protein SH groups in the liver and kidneys may be one of the mechanisms that leads to lipid peroxidation in these organs.

Keywords: cadmium, ethanol, glutathione, metallothionein, SH groups.

Introduction

Environmental pollution with toxic substances, e. g. heavy metals, is a serious problem. Cadmium (Cd) is one of the most toxic heavy metals. Occupational exposure to cadmium is common due to its wide industrial application. Uncontrolled Cd emissions from industrial sources produces a threat to the health of the general population [1, 2]. Smoking is a major source of exposure to Cd in the general population [1, 3], excessive alcohol consumption being

* Corresponding author: toxic@amb.edu.pl

another health-threatening problem. As ethanol (EtOH) abusers can also be occupationally or environmentally exposed to Cd, interactions between these two substances are an important epidemiological and medical issue.

Both Cd and EtOH exert nephro- and hepatotoxic effects. The lesions are to a certain degree related to the induction of cell membrane lipid peroxidation by these substances, which has been confirmed in our previous study [4, 5] and by other authors [6, 7, 8, 9, 10, 11]. An increase in the concentration of malondialdehyde (MDA, lipid peroxidation index) and disorders in the enzymatic antioxidant barrier (superoxide dismutase SOD and catalase CAT) were observed in the liver and kidney of animals exposed to Cd or EtOH singly [5]. Although Cd and EtOH induce lipid peroxidation, combined exposure to these substances did not result in their synergistic action [5].

Despite a number of studies conducted so far, the knowledge of the mechanism of lipid peroxidation under the influence of Cd and EtOH, particularly at a combined exposure, is not sufficient. As SH groups are lipid peroxidation stimulating factors [12, 13], the present study on the development of peroxidative changes at a combined exposure to Cd and EtOH has focused on a likely participation of SH group-containing compounds, such as reduced glutathione (GSH) and metallothionein (Mt) in the mechanisms of peroxidative action of Cd and EtOH.

GSH plays a key role in the system of cell defence, being involved in detoxication of many xenobiotics through feedback reaction in the second phase of biotransformation [14, 15]. GSH also protects cells against oxidative stress [15, 16]. Its role in this process is directly associated with the presence of reduced SH groups. Both the exposure to Cd and to EtOH causes changes in the GSH level [17, 18].

Mt is a low-molecular protein, rich in cysteine residues, responsible for the maintenance of zinc (Zn) and copper (Cu) homeostasis in the organism, and for detoxication of heavy metals, including Cd [19, 20, 21]. Mt synthesis is increased not only following exposure to heavy metals but also due to the action of other factors that induce oxidation disturbances [22, 23, 24, 25]. It allows the assumption that Mt can, like GSH, act as an antioxidant and free radical scavenger.

As the liver and kidneys, which play important roles in Cd and EtOH biotransformation and detoxication processes and can be damaged by these substances, exhibit the highest levels of SH groups, we decided to determine the concentrations of GSH, Mt and SH groups in these organs both after separate and combined administration of these two substances. The findings can help elucidate the mechanisms of interactions between these compounds in the process of lipid peroxidation, assessed previously in the same experimental model [5].

Experimental Procedures

Thirty-two adult (8-week-old) male Wistar rats, initial b. wt. 170 g, were used in the study. The animals were kept in standard breeding conditions (22 ± 2°C, relative humidity 50 ± 10%, natural 24 h cycle) and had unlimited access to drinking water and standard LSM diet (Agropol, Motycz, Polska). The energetic value of the diet was 12.2 MJ/kg. Cd concentration (determined in our laboratory) in the fodder was 0.122 μg/g.

The experiment lasted for 12 weeks. The rats were randomly divided into 4 groups of 8 animals each:

(1) control group, divided into two subgroups, of which one received Cd-free and EtOH-free redistilled water; the animals of the other were additionally given physiological saline (0.9% NaCl) through an intragastric tube in the same way EtOH was administered in groups 3 and 4;
(2) Cd group — animals were given to drink an aqueous solution of cadmium chloride at a concentration of 50 mg Cd/dm^3 as the only liquid;
(3) EtOH group — rats drank redistilled water and received intragastrically 5 g EtOH/kg b. wt./24 h in a total dose divided into two equal doses (2.5 g/kg b. wt. each) for 5 days a week (the first dose was administered at 8 a. m., the other 6 h later);
(4) Cd + EtOH group, animals were exposed to Cd in drinking water (like the Cd group) and were given EtOH (like the EtOH group).

The experiments were approved by the Białystok Local Ethical Committee for Experiments on Animals.

After the experiment termination in barbiturate anaesthesia (intraperitoneal administration of 30 mg Vetbutal/kg b. wt.) the liver and kidneys were collected for investigation. The organs were directly washed in cold 0.9% NaCl and weighed. The biological material was not directly used for analysis but was frozen at -80°C. The concentrations of GSH, Mt and SH groups were determined in the liver and kidneys and correlated with the previously described MDA concentrations in these animals [5].

The applied model of rat exposure to Cd and EtOH corresponds to the natural exposure of humans to these xenobiotics. Cd concentrations in the blood and urine of rats continuously intoxicated with 50 mg Cd/dm^3 [5, 21, 26] are within a range of values noted in inhabitants of areas heavily contaminated with Cd or Cd workers [2, 27]. The intragastrical dosage of 5 g EtOH/kg b. wt. /24 h is equivalent to consumption of about 0.7 l/day of 40% vodka in men [5, 10]. Since the rate of EtOH oxidation in rat is three times faster than in humans (0.1 g/kg b. wt./h), the animals need a higher dose of EtOH than humans to produce comparable toxic effects [10]. Thus, the level of EtOH treatment used in this study may be tantamount to its misuse in people.

Analytical Procedures

Glutathione (GSH) Determination

The concentration of GSH in the 5% homogenates of liver and kidneys (prepared in 5% metaphosphoric acid, MPA) was measured using Bioxytech GSH-400 test

(OXIS, USA). The method involves two steps. The first step leads to the formation of substitution products between a patented reagent and all mercaptans (RSH) present in the sample. The second step specifically transforms the substitution products obtained with GSH into a chromophoric thione whose maximal absorbance wavelength is 400 nm.

Metallothionein (Mt) Determination

The concentration of Mt in liver and kidney homogenates was assayed colorimetrically using the Micro Mt spec kit (Ikzus Environmental, Italy). The assay is based on the chemical determination of cysteine residues by Ellman's reaction [28]. Endogenous thiols such as GSH, free cysteine, etc. do not interfere with the assay.

The homogenates (0.5 g tissue with the addition of 1.5 ml homogenating buffer) were centrifuged at 30,000 x g for 20 min at 4°C. Then, after obtaining a supernatant, metallothionein was precipitated with alcohol, resuspended in denaturing buffer and chemically reacted with Ellman's reagent Bis (3-carboxy-4-nitrophenyl) disulfide. The absorption was read at 412 nm.

To obtain the concentration of Mt (nmol Mt) per gram of tissue, the following formula was applied:

$$\text{nmol Mt} \times \text{g}^{-1} = \frac{\text{nmol Cys}^{\text{Mt}}}{0.1\text{g} \times \text{n}^{\text{cys}}}$$

in which 0.1 g is the amount of tissue equivalent to 0.3 ml of supernatant subjected to precipitation; n^{cys} is the number of cysteine residues present in the investigated Mt (20 for most mammals); nmol Cys^{Mt} is the concentration of sulfhydryl groups, i. e. cysteine residues, due to metallothionein present in the sample.

Sulfhydryl Groups (SH) Determination

The concentrations of SH groups were estimated in protein and non-protein fractions (after deproteinization 10% of homogenates of liver and kidney prepared in 0.9% NaCl) according to Ellman [29]. Results are expressed as mmol/g tissue and μmol/g tissue.

Statistical Analysis

Since there were no differences in any of the studied parameters between the two control subgroups the results have been presented together as one-control group. Data are mean ± SEM of eight rats in each group. Experimental groups were compared using a one-way analysis of variance (ANOVA) by the Kruskal-Wallis ranks test. Spearman rank correlation analysis was performed to investigate the relationship among variables. Differences and correlations were considered statistically significant at $p < 0.05$. To discern the possible interactions between Cd and EtOH, two-way analysis of variance (ANOVA/MANOVA) was used. F values having $p < 0.05$ were considered significant. Statistical tests were performed using Statistica version 5.0 (StatSoft, Tulsa, OK, USA).

Results

GSH, Mt, Non-Protein SH and Total SH Concentrations in Liver

Exposure to Cd caused a reduction in the concentrations of GSH and non-protein SH groups compared to the control group (by 40% and 30.5%, respectively) ($p < 0.001$), while Mt concentration increased by 32.7% ($p < 0.001$) (Table 1). The concentration of total SH groups did not differ from control group.

In the animals exposed to EtOH a decrease was noted in the concentrations of GSH (by 44.7%, $p < 0.001$), non-protein SH groups (by 8.7%, $p < 0.05$) and total SH groups (by 9.6%, $p < 0.05$) compared to the control group. EtOH had no effect on Mt concentration (Table 1).

Combined exposure to Cd and EtOH caused a reduction in GSH concentration compared to the control group (by 50.1%, $p < 0.001$) and Cd group (by 16.8%, $p < 0.05$) (Table 1). Administration of EtOH to the animals which received Cd caused a reduction in the concentration of

Table 1. Effects of cadmium (Cd), ethanol (EtOH) and their combination on GSH, Mt, total SH and non-protein SH concentration in the liver.

Group	GSH (μmol/g tissue)	Mt (nmol/g tissue)	Non-protein SH (μmol/g tissue)	Total SH (mmol/g tissue)
Control	5.539 ± 0.110	47.86 ± 1.43	182.0 ± 2.615	7.939 ± 0.204
Cd	3.326 ± 0.147*	63.52 ± 1.66*	126.6 ± 3.206*	7.759 ± 0.232
EtOH	3.016 ± 0.114*	48.69 ± 1.54	166.4 ± 5.189*	7.180 ± 0.156*
Cd + EtOH	2.766 ± 0.202*†	56.73 ± 1.65*†‡	103.9 ± 3.512*†‡	7.823 ± 0.651‡

Values are means ± SEM of 8 rats. Values are significantly different (ANOVA + Kruskal-Wallis ranks test) compared to: *control, † Cd, and ‡ EtOH groups.

non-protein SH groups, both compared to the control and Cd or EtOH-receiving animals, by 43% ($p < 0.001$), 17.9% ($p<0.01$) and 37.6% ($p<0.001$), respectively. The concentration of total SH groups was within the control range.

The ANOVA/MANOVA analysis revealed that the changes in concentration of the thiol compounds examined in the liver of the animals exposed to a combined action of Cd and EtOH were not only due to an independent effect of Cd (the effect was observed for GSH, Mt and non-protein SH groups) or EtOH (for GSH and non-protein SH groups), but also resulted from interactions between these substances (for GSH, Mt and total SH groups) (Table 2).

It was found that the concentrations of GSH and non-protein SH groups in the liver were negatively correlated with MDA levels (Table 3). However, positive correlation was observed between MDA concentration and Mt level in this organ. The concentration of non-protein SH groups was positively correlated with GSH, but negatively with Mt. No correlation was noted between the levels of total SH groups and the other parameters examined.

GSH, Mt, Non-Protein SH and Total SH Concentrations in Kidneys

Exposure to Cd caused a reduction in the concentration of GSH (by 58.6%, $p<0.001$) and non-protein SH groups (by 17.6%, $p<0.01$), with a simultaneous increase in the levels of Mt (by 28.3%, $p<0.001$) and total SH groups (16.1%, $p<0.01$) in kidneys (Table 4).

In rats exposed to EtOH, a decrease was noted in the levels of GSH and non-protein SH groups compared to the control group by 46.6% ($p<0.001$) and 27% ($p<0.01$), respectively. The concentrations of Mt and total SH groups were similar to control values (Table 4).

Table 2. F values calculated with ANOVA/MANOVA analysis for main effects Cd or EtOH and interactive effect Cd and EtOH in the liver.

ANOVA/ MANOVA	GSH (µmol/g tissue)	Mt (nmol/g tissue)	Non-protein SH (µmol/g tissue)	Total SH (mmol/g tissue)
Main effect of Cd	F=71.7 p=0.000	F=56.8 p=0.000	F=247.2 p=0.000	NS
Main effect of EtOH	F=105.5 p=0.000	NS	F=26.1 p=0.000	NS
Interactive effect of Cd and EtOH	F=41.9 p=0.000	F=5.9 p=0.022	NS	F=4.6, p=0.041

NS, not significantly different.

Table 3. Correlation coefficients for the studied parameters in liver.

Parameters	MDA	GSH	Mt	Non-protein SH	Total SH
MDA	-----	r = -0.413 p = 0.000	r = 0.554 p = 0.001	r = - 0.482 p = 0.005	r = - 0.118 p = 0.518
GSH		------	r = -0.188 p = 0.303	r = 0.589 p = 0.000	r = 0.173 p = 0.345
Mt			------	r = - 0.716 p = 0.000	r = 0.219 p = 0.227
Non-protein SH				------	r = -0.183 p = 0.315

Data are presented as correlation coefficients (r) and the level of statistical significance (p)

Table 4. Effects of cadmium (Cd), ethanol (EtOH) and their combination on GSH, Mt, total SH and non-protein SH concentration in kidney.

Group	GSH (µmol/g tissue)	Mt (nmol/g tissue)	Non-protein SH (µmol/g tissue)	Total SH (mmol/g tissue)
Control	4.743 ± 0.203	52.66 ± 0.74	55.00 ± 1.65	3.401 ± 0.085
Cd	1.963 ± 0.115*	67.59 ± 0.64*	45.33 ± 1.92*	3.949 ± 0.039*
EtOH	2.578 ± 0.178*	51.39 ± 0.35	40.14 ± 1.50*	3.536 ± 0.099
Cd + EtOH	1.906 ± 0.086*‡	58.71 ± 1.39*†‡	37.14 ± 1.47*†	4.265 ± 0.091*†‡

Values are means ± SEM of 8 rats. Values are significantly different (ANOVA + Kruskal-Wallis ranks test) compared to: *control, †Cd and ‡EtOH groups.

In the group of animals that underwent a combined action of Cd and EtOH, GSH concentration was reduced compared to the control (by 59.8%, $p < 0.001$) and EtOH groups (by 26.1%, $p < 0.01$), while the level of non-protein SH groups decreased compared to the control and Cd groups (by 67.5%, $p < 0.001$ and 18.1%, $p < 0.05$, respectively) (Table 4). Administration of EtOH to the Cd-exposed rats caused an increase in Mt concentration, compared to the control group (by 11.5%, $p < 0.01$) and to the EtOH group (by 14.2%, $p < 0.01$), although this effect was smaller than in the rats exposed to Cd alone (by 13.1%, $p < 0.01$) (Table 4).

Two-way analysis of ANOVA/MANOVA showed that the changes in the parameters examined in the kidneys of animals exposed to a combined action of Cd and EtOH were the result of an independent effect of Cd (in the assessment of GSH, Mt, non-protein and total SH groups) or EtOH (GSH, Mt, non-protein and total SH groups), as well as by interactions between them (Table 5)

Kidney MDA concentration was found to be negatively correlated with GSH concentration and positively correlated with Mt and total SH groups (Table 6). GSH concentration showed positive correlation with non-protein SH groups and was negatively correlated with Mt and total SH groups. Positive correlation was noted between the concentration Mt and total SH groups in the kidneys.

Discussion

The study assessed the levels of GSH, Mt and SH groups in the liver and kidneys of rats exposed both singly and in combination to Cd and EtOH. Moreover, the paper was aimed at finding out the possible connection of lipid peroxidation and these thiol compounds. For this purpose, the relationship between GSH, Mt and SH groups and previously determined MDA concentrations [5] was investigated.

The experiment revealed the significant role of Cd- or EtOH-induced alterations in GSH concentration in lipid peroxidation in the liver and kidneys. The involvement of GSH in redox processes may be related to the presence of SH groups [14, 15]. During these processes induced by prooxidants, the reduced SH groups in GSH are used to scavenge free radicals. As a result, oxygenated GSH form is produced (disulfide GSH — GSSG) and GSH is reduced [18, 30]. It can thus be assumed that GSH reduction in the liver and so a decrease in non-protein SH groups following EtOH administration may be due to the interaction between free radicals that are produced during its biotransformation and SH groups in GSH. The significant independent effect of EtOH on the level of GSH and non-protein SH groups noted in the kidney seems to indicate that EtOH can induce lipid peroxidation through its effect on GSH levels. GSH not only reacts with free radicals, but also forms conjugates with numerous substances, including heavy metals [14, 15, 16]. The reduced level of GSH, and thus of non-protein SH groups, in the liver and kidneys of rats exposed to Cd, may result from the high affinity of this metal to thiol groups [13, 31, 32]. The formation of Cd complexes with the substances that contain thiol groups causes a reduction in the level of these substances [31, 32, 33]. More pronounced changes in GSH under the effect of Cd vs. EtOH in the kidney, compared to the liver, may result from metabolic differences. EtOH

Table 5. F values calculated with ANOVA/MANOVA analysis for main effects of Cd or EtOH and interactive effect of Cd and EtOH in the kidneys.

ANOVA/ MANOVA	GSH (μmol/g tissue)	Mt (nmol/g tissue)	Non-protein SH (μmol/g tissue)	Total SH (mmol/g tissue)
Main effect of Cd	F=127.2 p=0.000	F=164.4 p=0.000	F=14.8 p=0.001	F=60.7 p=0.000
Main effect of EtOH	F=52.7 p=0.000	F=34.3 p=0.000	F=48.9 p=0.000	F=7.6 p=0.010
Interactive effect of Cd and EtOH	F=47.5 p=0.000	F=19.2 p=0.000	NS	NS

NS, not significantly different.

Table 6. Correlation coefficients for the studied parameters in kidneys.

Parameters	MDA	GSH	Mt	Non-protein SH	Total SH
MDA	-----	r = -0.542 p = 0.001	r = 0.448 p = 0.010	r = - 0.243 p = 0.181	r = 0.528 p = 0.002
GSH		------	r = -0.554 p = 0.001	r = 0.524 p = 0.002	r = - 0.733 p = 0.000
Mt			------	r = - 0.030 p = 0.872	r = 0.546 p = 0.001
Non-protein SH				------	r = - 0.496 p = 0.004

Data are presented as correlation coefficients (r) and the level of statistical significance (p).

is metabolized mainly in the liver and hence its harmful effects are visible in this organ. Cd accumulates in the kidney, which is a Cd critical organ.

The increase in Mt concentration due to Cd exposure, especially in the kidneys, can be caused by enhanced synthesis of this protein under the effect of Cd, which is its major inducer [34, 35]. Increased Mt synthesis in the kidney is a likely cause of changes in the concentration of total SH groups under the effect of Cd. Positive correlation between Mt level in the organs examined and MDA concentration allows the assumption that the increase in Mt concentration is a defensive response of the organism to Cd- and EtOH-induced lipid peroxidation. Other authors also have suggested that Mt synthesis can be induced in oxidative stress conditions. This protein can act as an antioxidant and scavenger of free radicals such as superoxide, hydroxyl and peroxyl radicals [22, 23, 36]. The mechanisms of the antioxidative action of Mt are not well known and are still being explored.

Statistical analysis has confirmed that changes in GSH and Mt concentrations in the liver and kidneys, and in the level of total SH groups in the liver of animals exposed simultaneously to Cd and EtOH, may result from independent actions of Cd and/or EtOH, but can also be caused by interactions between these substances. Low Mt concentrations in the liver and kidneys observed in rats exposed to Cd and EtOH jointly, compared to those receiving Cd alone, is consistent with lower accumulation of this heavy metal noted in these animals [5].

The analysis of numerical values of ANOVA/MANOVA (F) variance coefficients seems to indicate that the reduced GSH level in the liver of rats exposed to Cd and EtOH was mainly caused by the action of EtOH, while in the kidney by Cd. This organ-related differentiation of GSH changes in conditions of a combined exposure to Cd and EtOH may result, at least partly, from the production of free radicals due to EtOH biotransformation in the liver and Cd accumulation in the kidneys.

The present findings suggest that the Cd- and/or EtOH-induced reduction in GSH and non-protein SH groups in the liver and kidney can be a mechanism leading to lipid peroxidation in these organs exposed to these substances. The combined exposure to Cd and EtOH is associated both with an independent action of each of these substances and with their interactions.

References

1. ATSDR. Toxicological profile cadmium. Agency for Toxic Substances and Disease Registry, Atlanta, GA, **1999**.
2. WORLD HEALTH ORGANIZATION (WHO). Environmental Health Criteria, 134 Cadmium. IPCS, Geneva, **1992**.
3. BEM E. M., PIOTROWSKI J. K., TURZYŃSKA E. Cadmium, zinc and copper levels in the kidneys and liver of the inhabitants of north-eastern Poland. Pol. J. Occup. Med. Environ. **6**, 133, **1993**.
4. BRZÓSKA M. M., MONIUSZKO-JAKONIUK J., PIŁATMARCINKIEWICZ B., SAWICKI B. Liver and kidney function and histology in rats exposed to cadmium and ethanol. Alcohol Alcohol. **38**, 2, **2003**.
5. JURCZUK M., BRZÓSKA M. M., MONIUSZKO-JAKONIUK J., GAŁAŻYN-SIDORCZUK M., KULIKOWSKA-KARPIŃSKA E. Antioxidant enzymes activity and lipid peroxidation in liver and kidney of rats exposed to cadmium and ethanol. Food Chem. Toxicol. **42**, 429, **2004**.
6. STOHS S. J., BAGCHI D., HASSOUN E., BAGCHI M. Oxidative mechanisms in the toxicity of chromium and cadmium ions. J. Environ. Pathol. Toxicol. Oncol. **19**, 201, **2000**.
7. CASALINO E., CALZARETTI G., SBLANO C., LANDRISCINA C. Molecular inhibitory mechanism of antioxidant enzymes in rat liver and kidney by cadmium. Toxicology **179**, 37, **2002**.
8. SHAIKH Z. A., VU T. T., ZAMAN K. Oxidative stress as a mechanism of chronic cadmium-induced hepatotoxicity and renal toxicity and protection by antioxidants. Toxicol. Appl. Pharmacol. **154**, 256, **1999**.
9. THURMAN R. G., BRADFORD B. U., IIMURO Y., FRANKENBERG M. V., KNECHT K. T., CONNOR H. D., ADACHI Y., WALL C., ARTEEL G. E., RAIEIGH J. A., FORMAN D. T., MASON R. P. Mechanism of alcohol-induced hepatotoxicity: studies in rats. Front. Biosci. **4**, 42, **1999**.
10. WIŚNIEWSKA-KNYPL J. M., WROŃSKA-NOFER T. Biological markers of oxidative stress induced by ethanol and iron overload in rats. Int. J. Occup. Med. Environ Health **7**, 355, **1994**.
11. MONTOLIU C., VALLES S., RENAU-PIQUERAS J., GUERII C. Ethanol-induced oxygen radical formation and lipid peroxidation in rat brain. Effect of chronic ethanol consumption. J. Neurochem. **63**, 1855, **1994**.
12. LI W., KAGAN H. M., CHOU I. N. Alterations in cytoskeletal organization and homeostasis of cellular thiols in cadmium-resistant cells. Toxicol. Appl. Pharmacol. **126**, 114, **1994**.
13. HULTBERG B., ANDERSSON A., ISAKSSON A. Copper ions differ from other thiol reactive metals ions in their effects on the concentration and redox status of thiols in HeLa cell cultures. Toxicology **117**, 89, **1997**.
14. VINA J. R., SPEZ G. T., VINA J. The physiological functions of glutathione. In: Handbook of Free Radicals and Antioxidants in Biomedicine (eds.: Miquel J., Quintanilha A. T., Weber H.), CRC Press, Boca Raton, vol. II, pp. 121-132, **1989**.
15. REED D. J. Glutathione: Toxicological implications. Ann. Rev. Pharmacol. Toxicol. **30**, 603, **1990**.
16. MEISTER A. Glutathione, ascorbate, and cellular protection. Cancer Res. **54**, 1969, **1994**.
17. GARCIA-FERNANDEZ A. J., BAYOUMI A. E., PEREZ-PERTEJO Y., MOTAS M., REGUERA R. M., ORDÓNEZ C., BALANA-FOUCE R., ORDÓNEZ D. Alterations of the glutathione-redox balance induced by metals in CHO-K1 cells. Comp. Biochem. Physiol. **132**, 365, **2002**.
18. SCOTT R. B., REDDY K. S., HUSAIN K., SCHLORFF E. C., RYBAK L. P., SOMANI S. M. Dose response of ethanol on antioxidant defence system of liver, lung, and kidney in rat. Pathophysiology **7**, 25, **2000**.

19. MEHTA A., FLORA S. J. S. Possible role of metal redistribution, hepatotoxicity and oxidative stress in chelating agents induced hepatic and renal metallothionein. Food Chem. Toxicol. **39**, 1029, **2001**.
20. PARK J. D., LIU Y., KLAASSEN C. D. Protective effect of metallothionein against the toxicity of cadmium and other metals. Toxicology **163**, 93, **2001**.
21. BRZÓSKA M. M., MONIUSZKO-JAKONIUK J. JURCZUK M., GAŁAŻYN-SIDORCZUK M. Cadmium turnover and changes of zinc and copper body status of rats continuously exposed to cadmium and ethanol. Alcohol Alcohol. **37**, 213, **2002**.
22. SATO M., BREMNER I. Oxygen free radicals and metallothionein. Free Rad. Biol. Med. **14**, 325, **1993**.
23. NATH R., KUMAR D., TIMAO L., SINGAL P. K. Metallothionein, oxidative stress and the cardiovascular system. Toxicology **155**, 17, **2000**.
24. SATO M., KONDOH M. Recent studies on metallothionein: protection against toxicity of heavy metals and oxygen free radicals. Tohoku J. Exp. Med. **196**, 9, **2002**.
25. ZHOU Z., SUN X., KANG Y. J. Metallothionein protection against alcoholic liver injury through inhibition of oxidative stress. Exp. Biol. Med. **227**, 214, **2002**.
26. BRZÓSKA M. M., KAMIŃSKI M., SUPERNAK-BOBKO D., ZWIERZ K., MONIUSZKO-JAKONIUK J. Changes in the structure and function of the kidney of rats chronically exposed to cadmium. I. Biochemical and histopathological studies. Arch. Toxicol. **77**, 344, **2003**.
27. CHALKLEY S. R., RICHMOND J., BARLTROP D. Measurement of vitamin D3 metabolites in smelter workers exposed to lead and cadmium. Occup. Environ. Med. **55**, 446, **1998**.
28. VIARENGO A., PONZANO E., DONDERO F., FABBRI R. A simple spectrophotometric method for metallothionein evaluation in marine organisms: an application to mediterranean and antarctic molluscs. Mar. Environ. Res. **44**, 69, **1997**.
29. ELLMAN G. L. Tissue sulfhydryl groups. Arch. Biochem. Biophys. **82**, 70, **1959**.
30. OH S. I., KIM CH. I., CHUN H. J., PARK S. CH. Chronic ethanol consumption affects glutathione status in rat liver. J. Nutr. **128**, 758, **1998**.
31. FIGUEIREDO-PEREIRA M. E., YAKUSHIN S., COHEN G. Distribution of the intercellural sulfhydryl homeostasis by cadmium-induced oxidative stress leads to protein thiolation and ubiquitination in neuronal cells. J. Biol. Chem. **273**, 12703, **1998**.
32. RIKANS L. E., YAMANO T. Mechanisms of cadmium-mediated acute hepatotoxicity. J. Biochem. Mol. Toxicol. **14**, 110, **2000**.
33. HAIDARA K., MOFFATT P., DENIZEAU F. Metallothionein induction attenuates the effects of glutathione depletors in rat hepatocytes. Toxicol. Sci. **49**, 297, **1999**.
34. WAALKES M. P., KLAASSEN C. D. Concentration of metallothionein in major organs of rats after administration of various metals. Fund. Appl. Toxicol. **5**, 473, **1985**.
35. KLAASSEN C. D., LIU J., CHOUDHURI S. Metallothionein: an intercellular protein to protect against cadmium toxicity. Annu. Rev. Toxicol. **39**, 267, **1999**.
36. KANG Y. J. The oxidant function of metallothionein in the heart. Proc. Soc. Exp. Biol. Med. **222**, 263, **1999**.