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Original Research

Evaluation of Oxidative Stress in Hepatic Mitochondria of Rats Exposed to Cadmium and Ethanol

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Abstract

The aim of our study was to evaluate oxidative stress in hepatic mitochondria of rats exposed for 12 weeks alone and in conjunction to cadmium (Cd) (50 mg Cd/dm^3, in drinking water) and ethanol (EtOH) (5g of EtOH/kg body wt, intragastrically). In hepatic mitochondria the concentrations of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), reduced glutathione (GSH) and the activities of manganese superoxide dismutase (MnSOD), glutathione peroxidase (GPx) and glutathione reductase (GR) were measured.

After exposure to Cd, an increase in MDA and H_2O_2 concentrations with a simultaneous decrease in GSH concentration and the activities of MnSOD, GPx and GR were noted. Exposure to EtOH caused an increase in MDA and H_2O_2 concentration, as well as MnSOD and GPx activities and a decrease in GSH concentration and GR activity. Co-exposure to Cd and EtOH caused an increase in MDA and H_2O_2 concentration as well as in MnSOD activity and resulted in a decrease in GSH concentration as well as GPx and GR activities in comparison to the control group. The increase in H_2O_2 concentration and MnSOD activity as well as the decrease in GSH concentration were significant compared to the animals exposed to Cd alone.

The changes noted in the investigated parameters in hepatic mitochondria of the rats co-exposed to Cd and EtOH resulted from an independent action of Cd (H_2O_2, GSH and GPx) or EtOH (H_2O_2, GSH and MnSOD) as well as from their mutual interaction (GSH, MnSOD and GPx).

Based on all results, it can be concluded that exposure to Cd and EtOH, both alone and in conjunction, leads mainly to changes in the concentration of H_2O_2 and GSH and MnSOD activity in the liver mitochondria.

Keywords: cadmium, ethanol, mitochondria, antioxidant enzymes, reduced glutathione, hydrogen peroxide, lipid peroxidation

Introduction

Cadmium (Cd) [1, 2] and ethanol (EtOH) [3, 4] belong to xenobiotics, having toxic action on the liver. The mechanisms of hepatotoxicity of these substances are complex and not completely explained. It is thought that one of these mechanisms might be oxidative stress. Acute and chronic exposure to EtOH induces oxidative-reductive disorders in the liver by the production of free radicals or the influence on the antioxidative system [3, 4, 5]. The role of Cd in oxidative stress induction consists mainly in the disorder of the antioxidative barrier in the organs [1, 6, 7]. Literature data give evidence that mitochondria can play a vital role in hepatotoxic action of Cd and EtOH [8-12].

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Mitochondria are essential for cell life by providing energy for cellular functions. In physiological conditions mitochondria are the major source of reactive oxygen forms (ROS). While producing energy necessary to convert ADP into ATP, mitochondria also produce superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$) as by products of the consumption of molecular oxygen in the electron transport chain [13, 14]. Mitochondria, susceptible to oxidation, possess a very effective antioxidative system [13-15]. The accumulation $O_2^{\bullet-}$ is eliminated by manganese superoxide dismutase (MnSOD), which generates H_2O_2 [13, 16]. Because mitochondria do not contain catalase (CAT), the mitochondrial redox system, which consists of reduced glutathione (GSH), glutathione peroxidase (GPx) and glutathione reductase (GR), plays a crucial role in reduction of H_2O_2 and protects mitochondria against peroxidative stress [15, 17, 18]. Disorders in the oxidative-reductive status of mitochondria leading to changes of a structure and function of these organelles, may be caused by numerous factors, including chronic consumption of EtOH [4, 11, 12] as well as environmental exposure to toxic substances such as Cd [8, 9].

In our studies [6, 19] it was revealed that one of the mechanisms of hepatotoxic action of Cd and EtOH in conditions of exposure alone and in conjunction are peroxidative disorders. Changes in the activity of superoxide dismutase (SOD), CAT and a decrease in GSH concentration in the liver play a significant role in induction of these disorders. Taking into account participation of mitochondria in Cd and EtOH hepatotoxicity, in the present study oxidative stress in mitochondrial fraction in the liver of rats exposed to Cd and/or EtOH was estimated. The concentration of malondialdehyde (MDA, an indicator of lipid peroxidation), H_2O_2 and GSH and the activity of MnSOD, GPx and GR were measured. This study was acknowledged to be purposeful since in the available literature there is no data on the oxidative stress in hepatic mitochondria at simultaneous exposure to Cd and EtOH.

Experimental Procedures

Animals and Treatment

Adult male Wistar rats (8 weeks old, weighing approximately 170 g) were used. The animals were kept under controlled, conventional conditions (temperature 22±2°C, relative humidity of 50±10%, natural day/night cycle) and had free access to drinking water (redistilled water free of contaminants) and standard LSM dry chow (Agropol, Motycz, Poland). The energetic value of the diet was 12.2 MJ/kg. Cd concentration (assessed in our laboratory) in the food was 0.122 μg/g. The experiment lasted for 12 weeks. The rats were randomly divided into four groups of 8 animals in each:

- The control group was divided into two subgroups; rats of the one were given redistilled water free of Cd and EtOH ad libitum, the animals of the other additionally received physiological saline (0.9% NaCl) p.o. (intragastrically through a tube);
- Cd group, which received an aqueous solution of cadmium chloride at a concentration of 50 mg Cd/dm^3 as the only drinking fluid;
- EtOH group, which received redistilled water to drink and 5 g EtOH/kg body wt./24 h, p.o in two equal doses of 2.5 g/kg body wt. each (the first dose was administered at 8 a.m., the other 6 h later) for 5 consecutive days a week during the whole experimental period;
- Cd + EtOH group, co-exposed to Cd (like the Cd group) and EtOH (like the EtOH group).

EtOH was applied in the form of 40% solution. To administer the dose of 5 g/kg body wt./24 to each rat, the volume of the EtOH solution was calculated individually for each of the animals of the EtOH and Cd + EtOH groups taking into consideration changes in body weight during the experimental period. Body weight of rats was monitored the first day of each week and on this basis appropriate volume of the 40% EtOH solution for each rat was calculated. The experimental model used here ensured equal EtOH and Cd intake in the groups of rats exposed to both substances alone and in conjunction with each other.

After the termination of the experiment, following overnight starvation, the rats were sectioned under barbiturate anaesthesia with Vetbutal (30 mg/kg body wt., i.p.). The liver was collected for investigation. The organ was directly washed in ice-cold 0.9% NaCl and weighed. The livers not used immediately were frozen at -80°C for further analysis. The concentrations of MDA, GSH and H_2O_2 as well as activities of MnSOD, GPx and GR were determined in hepatic mitochondria.

The experimental design was approved by the Local Ethics Committee for Animal Experiments in Bialystok (Poland) for care and use of laboratory animals.

The applied model of rats' exposure to Cd and EtOH corresponds to the exposure to these xenobiotics that may take place in human life. The exposure of rats to 50 mg Cd/dm^3 corresponds to human (especially smokers) environmental exposure to this toxic metal in heavily contaminated areas or in occupational conditions [20, 21]. The dose of 5 g EtOH/kg body wt./24 h is equivalent to the consumption by people of about 0.7 l/day 40% vodka [22]. Since the rate of EtOH oxidation in rats is three times faster than in humans (0.1 g/kg body wt./h), the animals need a higher dose of EtOH than humans to produce comparable toxic effects [22]. Thus the level of EtOH treatment used in this study may be tantamount to its misuse by humans.

Mitochondria Isolation and Assays

Mitochondrial fraction was prepared according to the procedure of Beattie et al. [23]. The slices of liver were homogenized in cold 0.9% NaCl (10% homogenate for GSH, MDA and H_2O_2), in 50 mM TRIS-HCl buffer,

pH 7.5, containing 5 mM EDTA and 1 mM dithiothreitol (8% homogenate for GPx), in 50 mM potassium phosphate buffer, pH 7.5, containing 1 mM EDTA (10% homogenate for GR) or in 20mM HEPES buffer, pH 7.2, containing 1 mM EGTA, 210 mM mannitol and 70 mM sucrose (5% homogenate for MnSOD), respectively. The homogenates were centrifuged at 800xg for 10 minutes at 4°C. Next, the supernatants were centrifuged at 8500xg for 20 minutes, and the resulting mitochondrial pellets were washed twice and reconstituted in the same buffers.

MDA was measured by the method of Buege and Aust [24]. The colour was measured spectrophotometrically at 532 nm. The concentration of MDA was expressed as nmol/g tissue.

H_2O_2 concentration was determined spectrophotometrically at 560 nm by a commercial kit (Bioxytech H_2O_2-560, OxisResearch, USA). Results were expressed as µmol/g tissue.

MnSOD was determined spectrophotometrically by Superoxide Dismutase Assay Kit (Cayman Chemical Company, USA). The absorbance was read at 450 nm using a plate reader. Activity of MnSOD was presented as mU/mg protein.

Activities of GPx and GR were measured using Bioxytech GPx-340 and Bioxytech GR-340 kits (OxisResearch, USA), respectively. Results were expressed as mU/mg protein.

The concentration of GSH was estimated by the colorimetric method using the Bioxytech GSH-400 kit (OxisResearch, USA). GSH level was presented as nmol/mg protein.

Mitochondrial protein concentration was determined by modified Lowry method [25] using bovine serum albumin as a standard.

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. Values are mean ± S.E. of eight rats in each group. The statistical calculations were made using a Statistica 5.0 package (StatSoft, Tulsa, OK, USA). To evaluate statistically significant differences between experimental groups, the Kruskal-Wallis one-way ANOVA was used. Spearman rank correlation analysis was performed to investigate the relationship among the studied parameters. Differences and correlations were considered statistically significant at $p < 0.05$. For possible interactions between Cd and EtOH, two-way analysis of variance (ANOVA/MANOVA, test F) was used. F values having $p<0.05$ were considered significant. In order to show a statistically significant difference in the studied parameters between the group of rats co-exposed to Cd and EtOH and the groups exposed to these xenobiotics separately, additional calculations were done with a view to defining the possible character of the interaction demonstrated in the ANOVA/MANOVA analysis. Then we compared the sum of the effects of Cd and EtOH action after separate exposure (Cd effect + EtOH effect) with that obtained in the experiment effect of the action of these xenobiotics after simultaneous exposure (Cd + EtOH effect). The effects of Cd and EtOH were presented as a percentage of changes of the studied parameters compared to control group. On the basis of the obtained difference it was estimated whether the interaction might have the synergistic (Cd + EtOH effect > Cd effect + EtOH effect), antagonistic (Cd + EtOH effect < Cd effect + EtOH effect) or dependent effect (Cd + EtOH effect ≠ Cd effect + EtOH effect) [26].

Results

MDA and H_2O_2 Concentrations in Hepatic Mitochondria

The exposure to Cd alone caused an increase in MDA and H_2O_2 concentrations in hepatic mitochondria in comparison to the control group, respectively by 7% ($p < 0.05$) and 10% ($p < 0.01$) (Fig. 1). After the exposure to EtOH, an increase in the concentration of MDA and H_2O_2 was noted compared to the control group by 12% ($p < 0.01$) and 53% ($p < 0.001$), respectively. Simultaneous exposure to Cd and EtOH caused an increase in MDA concentration by 9% ($p < 0.01$) compared to the control group. H_2O_2 concentration in the mitochondria increased compared to the control group by 79% ($p < 0.001$), to Cd group by 62% ($p < 0.001$) and to EtOH group by 17% ($p < 0.01$) (Fig. 1).

Analysis ANOVA/MANOVA revealed that observed change of H_2O_2 concentration in hepatic mitochondria of the rats exposed simultaneously to Cd and EtOH resulted from an independent action of Cd and EtOH and from their mutual interactions. The interaction between Cd and EtOH in relation to H_2O_2 had a synergistic character (Table 1). The change of MDA concentration in the Cd + EtOH group was not statistically significant compared to Gd and EtOH groups. Therefore, we did not estimate the possible character of the interaction between Cd and EtOH.

MDA concentration in the hepatic mitochondria positively correlated with H_2O_2 concentration ($r = 0.553$, $p = 0.001$).

MnSOD, GPx and GR Activities and GSH Concentration in Hepatic Mitochondria

After the exposure of rats to Cd only a decrease in the activities of MnSOD, GPx and GR, and GSH concentration were noted in mitochondria in comparison to the control group, by 36% ($p < 0.001$), 10% ($p < 0.05$), 16% ($p < 0.05$) and 28% ($p < 0.01$), respectively (Fig. 2). Exposure to EtOH caused an increase in MnSOD activity by 17% ($p < 0.05$) and GPx activity by 11% ($p < 0.05$), and a decrease in GR activity by 22% ($p < 0.01$) and GSH

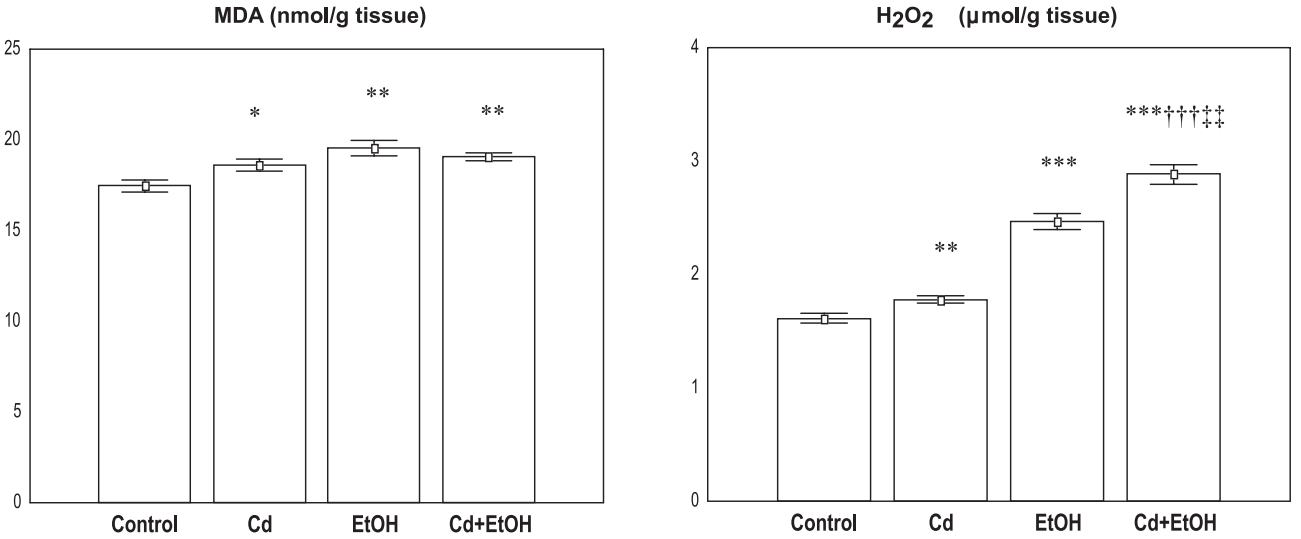

Fig.1. Effects of cadmim (Cd), ethanol (EtOH) and their co-exposure on malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) concentrations in the hepatic mitochondria.
Values are means ± S.E. for 8 rats. Statistically significant differences (Kruskal-Wallis one-way ANOVA) are indicated by * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. control; ††† $p < 0.001$ vs. Cd; ‡‡ $p < 0.01$ vs. EtOH group.

Table 1. Interactive effects of cadmium (Cd) and ethanol (EtOH) action regarding to the concentration of hydrogen peroxide (H_2O_2), reduced glutathione (GSH) and the activity of manganese superoxide dismutase (MnSOD), glutathione peroxidase (GPx) in the hepatic mitochondria.

	H_2O_2	GSH	MnSOD	GPx
ANOVA/MANOVA analysis of interactive effect of Cd and EtOH				
Main effect of Cd	F = 21.534 p = 0.000	F = 4.552 p = 0.042	NS	F = 41.272 p = 0.000
Main effect of EtOH	F = 244.58 p= 0.000	F = 142.64 p = 0.000	F =82.774 p = 0.000	NS
Interactive effect of Cd and EtOH	F = 4.580 p= 0.030	F = 22.571 p = 0.000	F = 32.557 p = 0.000	F = 7.589 p = 0.010
Estimation of the character of the interaction between Cd and EtOH				
Character of interaction	Cd + EtOH effect > Cd effect + EtOH effect Synergistic effect	Cd + EtOH effect ≠ Cd effect + EtOH effect Dependent effect (increased to Cd, opposed to EtOH)	Cd + EtOH effect ≠ Cd effect + EtOH effect Dependent effect (opposed to Cd, increased to EtOH)	Cd + EtOH effect ≠ Cd effect + EtOH effect Dependent effect (unchanged to Cd, opposed to EtOH)

F – the coefficient calculated with ANOVA/MANOVA; NS, not statistically significant effect

concentration by 68% ($p<0.001$) compared to control rats. After the simultaneous exposure to Cd and EtOH, the mitochondrial activity of MnSOD increased compared both to the control group and groups exposed separately to Cd and EtOH, by 40% ($p < 0.01$), 120% ($p < 0.001$) and 19% ($p < 0.05$), respectively. The GPx activity in the Cd + EtOH group decreased by 13% ($p < 0.01$) compared to the control group and by 22% ($p < 0.001$) compared to the EtOH group. The co-exposure to Cd and EtOH led to a decrease in GR activity in the mitochondria by 16% ($p < 0.05$) compared to control rats. GSH concentration in the hepatic mitochondria in the Cd + EtOH group decreased compared to the control group by 58% ($p < 0.001$) and compared to the Cd group by 41% ($p < 0.001$), whereas it increased compared to the EtOH group by 34% ($p < 0.05$) (Fig 1.)

Two-way analysis of variance ANOVA/MANOVA revealed that changes in the activity of the investigated enzymes and GSH concentration in the hepatic mitochondria of rats exposed simultaneously to Cd and EtOH resulted from an independent action of Cd (GPx and GSH) and EtOH (MnSOD and GSH) and their mutual interaction (MnSOD, GPx and GSH). The revealed interactions for GSH, MnSOD and GPx between Cd and EtOH had a complex character. The change observed in GSH concentration in rats co-exposed to Cd and EtOH

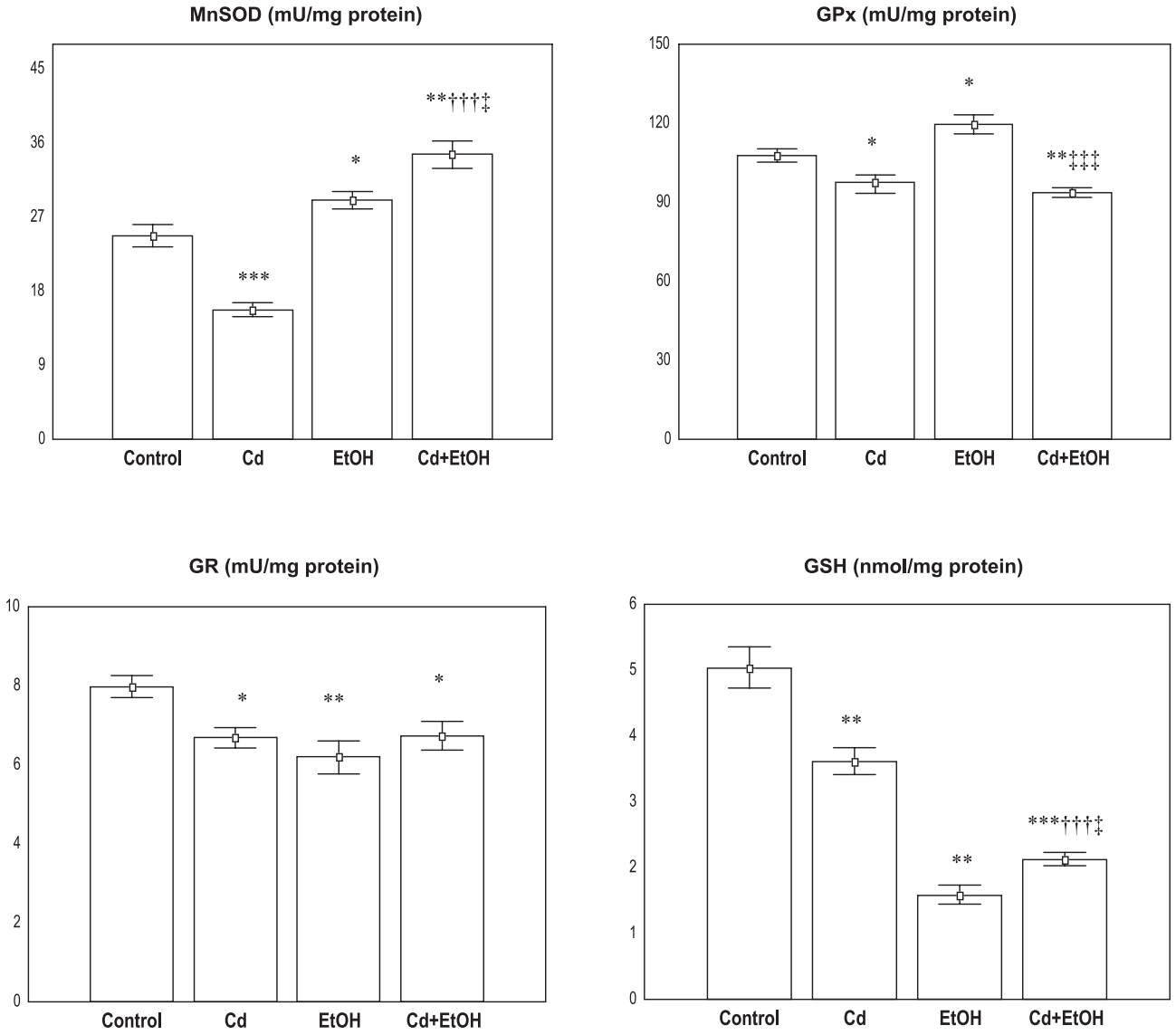

Fig.2. Effects of cadmim (Cd), ethanol (EtOH) and their co-exposure on manganese superoxide dismutase (MnSOD), glutathione peroxidase (GPx), glutathione reductase (GR) activity and reduced glutathione (GSH) concentration in the hepatic mitochondria.
Values are means ± S.E. for 8 rats. Statistically significant differences (Kruskal-Wallis one-way ANOVA) are indicated by * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs. control; ††† $p<0.001$ vs. Cd; ‡ $p<0.05$, ‡‡‡ $p<0.001$ vs. EtOH group.

was increased compared to the Cd group and it was opposed compared to the EtOH group. In the case of MnSOD, the interaction opposed was observed between Cd and EtOH compared to Cd action and increased compared to the EtOH effect. The GPx activity observed in the Cd + EtOH group, in the demonstrated interaction, did not change in comparison to the Cd group but was decreased compared to the EtOH group (Table 1). The change of the activity GR in the Cd + EtOH group was not statistically significant compared to Cd and EtOH groups; therefore, the possible character of the interaction between Cd and EtOH was not estimated.

A positive correlation was revealed (r=0.595, p=0.001) between MnSOD activity and H_2O_2 concentration in the hepatic mitochondria. GSH concentration in mitochondria was negatively correlated with MDA concentration (r= –0.617, p=0.000), H_2O_2 (r= –0.783, p=0.000) and MnSOD activity (r= –0.540, p=0.001), and positively correlated with GR activity (r=0.450, p=0.010). GR activity was negatively correlated with H_2O_2 concentration (r= –0.365, p=0.040).

Discussion

Mitochondria play a vital role in regulation of cell oxidative stress [10, 13, 14]. They generate ROS and simultaneously are very sensitive to its damaging action. Our [6, 19] and other authors' [2-4, 7] studies give evidence that in the toxic action of Cd and EtOH on a liver the sig-

nificant part has oxidative stress induced by these xenobiotics. Because literature data indicate that mitochondria are an important target of the toxic action of Cd [9, 27, 28] and EtOH [11, 12, 29] and that oxidative changes taking place in cell organelles can play a vital role in hepatotoxic action of Cd and EtOH [8-12], in the present study the oxidative stress was estimated in hepatic mitochondria of rats, in which exposure alone and in conjunction to these xenobiotics cause peroxidative disorders in this organ. In hepatic mitochondria concentrations of H_2O_2, MDA, GSH and activities of MnSOD, GPx and GR were measured.

Exposure to Cd caused a significant decrease in MnSOD activity in the hepatic mitochondria. This enzyme, as it was mentioned in the Introduction, is used in the reaction of dismutation of $O_2^{\bullet-}$ to H_2O_2. Literature data report that the main reason for this radical generation in the mitochondria is disturbed electron transport through the complex I (NADH: ubiquinone oxidoreductase) and complex III (ubiquinone: cytochrome c oxidoreductase) in the electron transport chain [13, 16]. Studies by Wang et al. [30] demonstrated that Cd can inhibit complex III in hepatic mitochondria and induce ROS production in this complex. Therefore, the decrease in MnSOD activity noted in the mitochondria of rats exposed to Cd might result from using this enzyme in the reaction of dismutation of $O_2^{\bullet-}$. However, other mechanisms leading to the decrease in MnSOD activity due to exposure to Cd cannot be excluded. Casalino et al. [1] proved that Cd caused the inhibition of MnSOD activity in the liver and kidney mitochondria by replacing Mn ions. They have not observed any changes in MDA concentration. In the present study, the decrease in MnSOD activity was accompanied by the simultaneous increase in H_2O_2 and MDA concentration. H_2O_2, generated in reaction of dismutation, catalyzed by MnSOD, can reach plasma membrane and takes part in oxidative modification of its components [31]. The increase in MDA concentration in the mitochondria of rats exposed to Cd suggests that lipid peroxidation was stimulated as a result of increased H_2O_2 concentration. Literature data give evidence that a decrease in the mitochondria GSH concentration may play a crucial role in hepatotoxic action of Cd [8, 9]. The mechanism of the decrease in the mitochondrial GSH concentration due to Cd is not completely known. It is assumed that the level of this tripeptide in the mitochondria can be decreased as a result of increased activity of gamma-glutamyltransferase (GGT), the membrane enzyme responsible for breakdown of GSH [32], or destruction of availability of NADPH necessary for proper GR action [33]. In the present study the decrease of GR and GPx activity in hepatic mitochondria after exposure to Cd was noted. Because GSH is a specific cofactor for GPx we can assume that the decrease in the activity of this enzyme may result from the decrease in the mitochondrial GSH concentration. More complete explanation of the relationship between GSH concentration and activity of GPx and GR in hepatic mitochondria and the participation of this redox system in H_2O_2 reduction during exposure to Cd requires further investigation.

The changes of the measured parameters of oxidative status in hepatic mitochondria of rats exposed to EtOH were more intensified than those at the exposure to Cd. Exposure to EtOH caused an almost 5-fold increase in H_2O_2 concentration and 2.5–fold decrease in mitochondrial GSH concentration compared to exposure to Cd. The activity of MnSOD and GPx after exposure to EtOH, as opposed to the exposure to Cd, increased. In properly functioning mitochondria to eliminate H_2O_2 the balance exists between MnSOD activity (the enzyme responsible for H_2O_2 production) and GPx (the enzyme which metabolises H_2O_2) [10]. In basic aerobe conditions, with an increase in MnSOD activity the activity of GPx increases as well. Despite the fact that after exposure to EtOH this trend in changes of enzyme activities was observed in hepatic mitochondria, the enormous increase in H_2O_2 concentration can suggest an improper relationship between MnSOD and GPx in these organelles. H_2O_2 can accumulate in mitochondria if GPx activity is lower than MnSOD. The mechanisms of toxic action of EtOH on both MnSOD and GPx activity are not recognized. Depending on conditions both increase and lack of influence of EtOH on MnSOD activity were noted [34]. Similarly, not completely known is the influence of this xenobiotic on GPx activity in mitochondria [35]. Because the significant decrease in mitochondrial GSH concentration was obtained we can assume that this could be the reason for lower GPx activity, and thus the increase in H_2O_2 concentration. One of the important functions of mitochondrial GSH is controlling the endogenous H_2O_2 formation during mitochondria electron transport through the respiratory chain [10]. There are significant stechiometric differences between the pool of mitochondrial GSH and H_2O_2 level in mitochondria matrix (in proper conditions H_2O_2 concentration is 1000 times lower than GSH) [18] and significant decreases in mitochondrial GSH (50–60% of the control value) can importantly influence the detoxification of H_2O_2 [18]. GSH is not synthesized in mitochondria. The pool of mitochondrial GSH, being about 10–15% of the whole cell content of GSH, is determined by activity of specific transporters, located in the internal mitochondria membrane, which transmit GSH from cytosol to internal mitochondria matrix [36]. Literature data reveal that chronic exposure to EtOH causes a decrease in mitochondrial GSH as a result of disturbed uptake of GSH from a cytosol [10, 37, 38]. It can be assumed that disturbed (as a result of action of EtOH) uptake of GSH from a cytosol to mitochondria can be the reason for such marked (68%) reduction of mitochondrial GSH in this experiment.

Co-exposure to Cd and EtOH caused high increase in H_2O_2 concentration in the mitochondria, which was also significant in relation to separate exposure to these xenobiotics. Two-way analysis ANOVA/MANOVA revealed that the change of this parameter resulted from an independent action of Cd and EtOH. However, acquired high values of F factor for EtOH can suggest that EtOH has the most influence on this parameter. The high negative corre-

lation between H_2O_2 concentration and GSH concentration and high positive correlation between H_2O_2 concentration and MnSOD activity in the hepatic mitochondria can suggest that changes in mitochondrial GSH concentration and MnSOD activity noted after the co-exposure to Cd and EtOH determine such high H_2O_2 induction in these organelles. Similarly to H_2O_2, EtOH has the main contribution in decreasing mitochondrial GSH and in increasing MnSOD activity. We cannot exclude, based on conducted two-way analysis, participation of Cd and mutual interactions between this metal and EtOH in changes of investigated parameters. The synergistic character of the interaction between Cd and EtOH for H_2O_2 and the dependent effects for GSH, MnSOD and GPx might be the reason why such different changes in the studied parameters at co-exposure were obtained. A more detailed explanation of the mechanisms of the influence of the interaction between Cd and EtOH on H_2O_2 and GSH concentration as well as on MnSOD and GPx activities requires further studies on the cell level.

On the basis of results we can hypothesize that after simultaneous exposure to Cd and EtOH, similarly like after separate exposure to these xynobiotics, main changes in hepatic rat mitochondria refer to H_2O_2 concentration, MnSOD activity and, first of all, GSH. Despite the positive correlation between H_2O_2 concentration in the mitochondria and MDA concentration in these organelles, the intensity of lipid peroxidation in the mitochondria was not enhanced after simultaneous exposure to Cd and EtOH in comparison to separate exposure to these xenobiotics. The study will be continued to find a more complete explanation of the role of mitochondrial H_2O_2 in hepatotoxic action of Cd and EtOH at co-exposure.

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