Evaluation of glutathione-related enzyme activities in the liver and kidney of rats exposed to lead and ethanol

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Abstract: Recently, we have put forward the hypothesis that a decreased concentration of reduced glutathione (GSH) may be implicated in the mechanisms of peroxidative damage to the liver and kidney caused by lead (Pb) and/or ethanol (EtOH). Thus, the aim of the present study was to assess the activities of GSH-related enzymes, such as glutathione peroxidase (GPx), glutathione reductase (GR), and glutathione S-transferase (GST) in these organs of rats exposed to Pb (500 mg/l in drinking water) and/or EtOH (5 g/kg/24 h intragastrically) for 12 weeks. The exposure to Pb led to a decrease in the hepatic activities of GPx, GR and GST and an increase in the renal activities of GPx and GR. After the exposure to EtOH, a decrease in the hepatic activities of GPx and GR and an increase in the renal GPx activity were observed. The co-exposure to Pb and EtOH resulted in a decrease in the activity of the study enzymes in the liver. The decrease in the hepatic GPx activity was also significant compared to the animals exposed to Pb and EtOH separately. The renal GR activity increased due to the co-exposure to Pb and EtOH in comparison with the control group and the groups treated with Pb and EtOH separately, whereas the renal GPx activity increased only compared to the control group. Analysis of variance revealed that the changes in the activity of the study enzymes after co-exposure to Pb and EtOH resulted from an independent action of these xenobiotics as well as from their interactive action. The results suggest that changes in GPx, GR and GST activities may belong to the mechanisms leading to a decrease in GSH concentration in the liver and kidney due to exposure to Pb and/or EtOH.

Key words: lead, ethanol, glutathione peroxidase, glutathione reductase, glutathione S-transferase

Introduction

Lead (Pb) and ethanol (EtOH) disturb numerous biochemical processes in the organism and damage various tissues and organs [6, 10, 14, 25, 39, 42, 48]. These effects result, among others, from their ability to induce oxidative stress [1, 9, 12, 39, 44, 47]. One of the mechanisms of the Pb- and EtOH-induced oxidative stress is disturbance in the homeostasis of reduced glutathione (GSH) [8, 16, 26, 27, 31]. GSH, which is a thiol compound, acts in the organism as an important low-molecular-weight antioxidant [8, 33], participating in the reduction of hydrogen peroxide \( \text{H}_2\text{O}_2 \) and organic peroxides formed during lipid peroxidation catalyzed by glutathione peroxidase (GPx, E.C. 1.11.1.9) [2, 33]. GSH can also react with hydroxyl radical, nitrogen oxide (III) and peroxynitrite in non-enzymatic reactions [5, 8, 15]. In GPx-catalyzed reactions, GSH is oxidized to a disulfide (GSSG), which – reduced by glutathione reductase...
The reaction occurs in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH). The properly functioning GR/GPx cycle is responsible for maintaining the GSH level in the cell [11].

GSH, through S-conjugate formation, is involved in the detoxification of many xenobiotics, including lipid peroxidation products [4, 23, 45]. The reactions of S-conjugation can be accelerated by glutathione S-transferase (GST, E.C. 2.5.1.18) [45].

Recently, we have revealed that both separate and combined exposure to Pb and EtOH resulted in a decrease in GSH concentration in the liver and kidney [19]. Moreover, we have found that the decrease in the liver GSH concentration was higher after co-exposure to Pb and EtOH than after the exposure to these xenobiotics separately. Based on our findings, we have concluded that Pb- and EtOH-induced decrease in the liver and kidney GSH concentration may be one of the mechanisms of their peroxidative action in the two organs, especially under co-exposure.

Literature data prove that both Pb [11, 27] and EtOH [6, 9, 31, 41] influence the activity of enzymes responsible for the maintenance of GSH homeostasis in the cells, such as GPx, GR and GST. However, there are no data concerning the influence of combined exposure to Pb and EtOH on the activity of these enzymes.

Thus, the aim of the present study was to estimate the influence of both separate and combined exposure to Pb and EtOH on the activities of GPx, GR and GST in the liver and kidney of the same rats in which a decrease in GSH concentration was determined [19]. Moreover, in order to explain the role of GPx, GR and GST in the Pb and EtOH-induced lipid peroxidation and in the decrease in GSH concentration in the liver and kidney, we evaluated the correlation between the activities of the study enzymes and previously reported concentrations of GSH, Pb and MDA, evaluated in the same rats [19, 20]. The assumption has been made that the results will contribute to an explanation of the mechanisms of disturbance in GSH homeostasis in the liver and kidney due to the exposure to Pb and EtOH, especially when given jointly.

**Materials and Methods**

**Animals**

Adult male Wistar rats (8-week-old weighing approximately 170 g) were used in our study. The animals throughout the experiment were kept under controlled conditions (temperature 22 ± 2°C, relative humidity of 50 ± 10%, natural day/night cycle) and had free access to drinking water and the standard LSM dry chow (Agropol, Motycz, Poland).

The experimental design was approved by the Local Ethic Committee for Animal Experiments in Białystok (Poland). Procedures involving the animals and their care conformed to the institutional guidelines and were in compliance with national and international laws and Guidelines for the Use of Animals in Biomedical Research.

**Experimental protocol**

The rats were randomly divided into four groups (control, Pb, EtOH and Pb + EtOH) of eight animals each. The control group was divided into two subgroups, of which one received redistilled water free of Pb and EtOH; the animals of the other were additionally given physiological saline (0.9% NaCl; intragastrically through a tube). The animals of the Pb group were administered an aqueous solution of lead acetate (Pb(CH₃COO)₂ × 3 H₂O; POCh, Gliwice, Poland) at the concentration of 500 mg Pb/l, as the only drinking fluid. The rats of the EtOH group drank redistilled water and received EtOH at a total dose of 5 g/kg/24 h divided into two equal doses of 2.5 g/kg each (the first dose was administrated at 8 a.m., the other 6 h later) for 5 days a week during the experiment. The Pb + EtOH group was exposed to Pb in drinking water (like the Pb group) and received EtOH (like the EtOH group).

The experiment lasted for 12 weeks. EtOH was applied in the form of 40% solution. The volume of 40% EtOH solution corresponding to a dose of 5 g/kg/24 h was calculated individually for each rat from the EtOH and Pb + EtOH groups, depending on changes in the body weight during the experiment. The rats were weighed on the first day of each week to modify the EtOH volume. The body weight, 24-h consumption of drinking water in each of the four experimental groups and Pb intake in the Pb and Pb + EtOH groups during the whole experiment have been presented in our previous studies carried out in these rats [19, 20]. Due to bad taste of water containing lead acetate, fluid consumption in the Pb and Pb + EtOH groups was lower compared to the control and EtOH groups [20].

The experimental model used in the study ensured equal EtOH and Pb intake in the groups of rats ex-
posed to both substances alone and in combination, which is very important in the evaluation of mutual interactions between two substances. Pb intake in the Pb and Pb + EtOH groups calculated on the basis of the 24-h consumption of drinking water during the whole course of experiment was within the following ranges 34.3–60.3 and 32.7–56.2 mg Pb/kg/24 h, respectively, of which 1–2% might be absorbed from the rat’s gastrointestinal tract [36]. The detailed data on the Pb intake have been recently reported in our previous study [20].

After the experiment, following overnight starvation, the rats were sectioned under barbiturate anesthesia with Vetbutal (30 mg/kg, ip, Biowet, Pulawy, Poland) and the liver and kidney were removed. The organs were directly washed in ice-cold 0.9% NaCl and weighed. The biological material not used immediately was frozen at –80 oC for further analysis. The activities of GPx, GR and GST in the liver and kidney of rats have already been reported in our previous studies [19, 20].

The liver and kidney activities of GPx, GR and GST were determined in the present study. Since there were no differences in any of the study parameters between the two control subgroups the results were presented together as one control group. Values are the mean ± SE of eight rats in each group. The Kruskal-Wallis one-way ANOVA was used to evaluate statistically significant differences between experimental groups. Spearman rank correlation analysis was performed to investigate the relationship among GPx, GR and GST activities. Moreover, correlations between the activity of these enzymes and GSH, Pb and MDA concentrations previously reported [19, 20] in those rats were estimated in our present study. Detailed data on GSH, Pb and MDA concentrations have recently been reported [19, 20].

Glutathione-related enzymes in rats exposed to lead and ethanol

Table 1. Effect of lead (Pb) and/or ethanol (EtOH) on Pb, reduced glutathione (GSH) and malondialdehyde (MDA) concentrations in the liver and kidney of rats 

<table>
<thead>
<tr>
<th>Group</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>↓↓</td>
<td>↑↑</td>
</tr>
<tr>
<td>EtOH</td>
<td>↑↑</td>
<td>↓↓</td>
</tr>
<tr>
<td>Pb + EtOH</td>
<td>↑↑†</td>
<td>↓↓†</td>
</tr>
</tbody>
</table>

a Detailed data have been reported earlier [19, 20]. † without statistically significant change (p < 0.05; Kruskal-Wallis one-way ANOVA). Statistically significant increase (↑) or decrease (↓) compared to * control, † Pb and ‡ EtOH group.
and in the present study, these results are only summarized in Table 1.

Differences and correlations were considered statistically significant at \( p < 0.05 \). Two-way analysis of variance (ANOVA/MANOVA, test F) was used to discern the possible interactions between Pb and EtOH. F values having \( p < 0.05 \) were considered statistically significant. All statistical calculations were made using Statistica 5.0 package (StatSoft, Tulsa, OK, USA).

## Results

In the rats exposed to Pb alone, the hepatic activities of GPx, GR and GST decreased by 18%, 29% and 12%, respectively, compared to the control group (Fig. 1). The exposure to EtOH alone resulted in a decrease in the hepatic activities of GPx (by 8%) and GR (by 24%), but had no influence on GST activity, compared to the control (Fig. 1). The co-exposure to Pb and EtOH led to a decrease in the hepatic activity of GPx (by 28%) compared to the control group (by 28%), as well as the groups treated with Pb (by 12%) and EtOH (by 22%) alone. The activity of GR was lower in the Pb + EtOH group only when compared to the control group (by 19%), whereas the hepatic GST activity decreased compared to the control and EtOH groups by 14% and 7%, respectively (Fig. 1).

The ANOVA/MANOVA analysis of variance revealed that the change in the hepatic GPx activity in the rats co-exposed to Pb and EtOH resulted from an independent action of the two xenobiotics. The hepatic GR activity in those rats was influenced by an independent action of Pb and its interaction with EtOH. The change in the hepatic GST activity was only Pb-dependent (Tab. 2).

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**Fig. 1.** Effects of lead (Pb), ethanol (EtOH) and their co-exposure on glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) activities in the liver of rats. Values are the mean ± SE for 8 rats. Statistically significant differences (Kruskal-Wallis one-way ANOVA) are indicated by * \( p < 0.05 \), ** \( p < 0.01 \) and *** \( p < 0.001 \) vs. control; * \( p < 0.05 \) vs. Pb group; ** \( p < 0.05 \) and *** \( p < 0.001 \) vs. EtOH group.

**Tab. 2.** F-values calculated with ANOVA/MANOVA for the main effect of lead (Pb) or ethanol (EtOH) and their interactive effect

<table>
<thead>
<tr>
<th>ANOVA/MANOVA</th>
<th>GPx (mU/mg protein)</th>
<th>GR (mU/mg protein)</th>
<th>GST (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of Pb</td>
<td>( F = 61.405 )</td>
<td>( F = 6.266 )</td>
<td>( F = 12.927 )</td>
</tr>
<tr>
<td></td>
<td>( p = 0.000 )</td>
<td>( p = 0.018 )</td>
<td>( p = 0.001 )</td>
</tr>
<tr>
<td>Main effect of EtOH</td>
<td>( F = 14.318 )</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>( p = 0.001 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interactive effect of Pb and EtOH</td>
<td>NS</td>
<td>( F = 12.662 )</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( p = 0.001 )</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main effect of Pb</td>
<td>( F = 4.301 )</td>
<td>( F = 55.985 )</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>( p = 0.047 )</td>
<td>( p = 0.000 )</td>
<td></td>
</tr>
<tr>
<td>Main effect of EtOH</td>
<td>( F = 18.154 )</td>
<td>( F = 22.057 )</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>( p = 0.000 )</td>
<td>( p = 0.000 )</td>
<td></td>
</tr>
<tr>
<td>Interactive effect of Pb and EtOH</td>
<td>( F = 8.525 )</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>( p = 0.007 )</td>
<td></td>
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</tr>
</tbody>
</table>

NS – without statistically significant effect; GPx – glutathione peroxidase, GR – glutathione reductase, GST – glutathione S-transferase.
The hepatic activities of GPx and GST correlated negatively with Pb and MDA concentrations, and positively with GSH concentration in this organ (Tab. 3). A positive correlation was also noted between the hepatic GPx activity and the activities of GR and GST.

The exposure to Pb alone caused an increase in the renal activities of GPx and GR by 28% and 10%, respectively, compared to the control group (Fig. 2). Administration of EtOH alone increased GPx activity by 34% compared to the control animals (Fig. 2). The activity of GPx was increased by 31% in the Pb + EtOH group compared to the control group (Fig. 2). The co-exposure to Pb and EtOH led to an increase in the renal GR activity compared both to the control group and groups exposed separately to Pb and EtOH, by 22%, 10% and 15%, respectively (Fig. 2). The renal GST activity was unchanged by any treatment (Fig. 2).

The ANOVA/MANOVA analysis revealed that the change in the renal GPx activity in the rats co-exposed to Pb and EtOH resulted from an independent action of the two xenobiotics as well as from the interaction between them. The renal GR activity in the Pb + EtOH group was influenced by an independent action of Pb and EtOH (Tab. 2).

The renal GPx activity correlated positively with the concentration of Pb and negatively with GSH concentration in this organ (Tab. 3). A positive correlation was also noted between the hepatic GR activity and the activities of GPx and GST.

The ANOVA/MANOVA analysis revealed that the change in the renal GPx activity in the rats co-exposed to Pb and EtOH resulted from an independent action of the two xenobiotics as well as from the interaction between them. The renal GR activity in the Pb + EtOH group was influenced by an independent action of Pb and EtOH (Tab. 2).

The renal GPx activity correlated positively with the concentration of Pb and negatively with GSH concentration in this organ (Tab. 4). Positive correlations were noted between the renal GR activity and the concentration of Pb and MDA, as well as the activity of GPx. Moreover, the renal activity of GR correlated negatively with GSH concentration.

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**Fig. 2.** Effects of lead (Pb), ethanol (EtOH) and their co-exposure on glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione S-transferase (GST) activities in the kidney of rats. Values are the mean ± SE for 8 rats. Statistically significant differences (Kruskal-Wallis one-way ANOVA) are indicated by **p < 0.01 and ***p < 0.001 vs. control; **p < 0.01 vs. Pb; ***p < 0.001 vs. EtOH group.
Discussion

Our previous findings [19] as well as few reports of other authors [12, 30, 31, 41, 47] suggest that both Pb—
and EtOH-induced decrease in GSH concentration in the liver and kidney may be one of the mechanisms of peroxidative action of Pb and EtOH in these organs. Enzymes, such as GPx, GR and GST, take part in maintaining GSH homeostasis in tissues. The knowledge of the influence of separate and combined exposure to Pb and EtOH on the activity of these enzymes may contribute to the explanation of the mechanisms responsible for a decrease in GSH concentration the liver and kidney due to the exposure to these xenobiotics. Thus, the aim of the present study was to evaluate the activities of GPx, GR and GST in the liver and kidney of rats co-exposed to Pb and EtOH.

The positive correlation between GSH concentration and GPx activity noted in the liver may suggest that a decrease in GPx activity is accompanied by a decrease in the concentration of this tripeptide. GPx is one of the most important antioxidant enzymes, which, together with GSH as a donor of electrons, catalyzes the reactions of H2O2 and lipid peroxide reduction [2, 33]. The experimental studies provide the evidence that both Pb [1, 12, 44] and EtOH [9, 41] generate reactive oxygen species (ROS), such as superoxide ion, hydrogen peroxide, hydroxyl radicals as well as products of lipid peroxidation, such as lipid hydroperoxides and lipid aldehydes. The decrease in GPx activity due to the exposure to Pb or EtOH could be connected with its action, together with GSH, as a scavenger of peroxides induced by these xenobiotics. A decline in GPx activity as a result of ROS inactivation caused by Pb [38] and EtOH [31] exposure was reported in the studies of other authors. Another mechanism that leads to the decrease in GPx activity after Pb exposure might be the inhibition of thiol groups (-SH) in the enzyme. Pb is characterized by high affinity for -SH groups, especially in cysteine, which in consequence leads to the development of mercaptides and to a decrease in the stability of cysteine complexes with other amino acids present in the chain [49]. Pb is able to inactivate -SH groups also in such enzymes as dehydratase of δ-aminolevulinic acid (δ-ALAD), SOD, CAT and glucose-6-phosphate dehydratase (G6PD) [7, 22]. Ethanol (or its metabolite – acetaldehyde) also seems to inhibit the activity of GPx by interacting with their nucleophilic groups [35]. As GPx is a selenoenzyme, a decreased intake of selenium may inhibit this enzyme activity. Experimental studies revealed that both Pb [40] and EtOH [29] might reduce selenium content in the organism.

Taking into account the results of ANOVA/MANOVA analysis of variance and percentage changes in GPx activity due to the exposure to Pb or/and EtOH, it may be assumed that the statistically significant decrease in the activity of this enzyme after the co-exposure to Pb and EtOH results from an independent action of both xenobiotics. The higher F value (ANOVA/MANOVA) for Pb, compared to F value for EtOH suggests that Pb has a bigger than EtOH influence on the GPx activity at the co-exposure to Pb and EtOH.

An oxidized GSH form, i.e. GSSG, is formed in the GPx-catalyzed reaction. GSSG is toxic for the cells, hence, a system consisting of GR and NADPH removes it from the cells and regenerates it to GSH [3, 26]. A decrease in GR activity or a decline in NADPH content inhibits GSH regeneration. Our study demonstrated a decrease in the hepatic GR activity due to the exposure to Pb and EtOH, both alone and in conjunction. The EtOH-caused decrease in GR activity might result from its involvement in the process of GSSG reduction that is formed in excessive amounts. Moreover, it should also be taken into consideration that the decrease in the GR activity might result from depletion of NADPH, which is a co-substrate required for this enzyme activity [8]. In conditions of chronic exposure to EtOH, the concentration of NADPH is decreased due to EtOH biotransformation [31]. G6PD is responsible for the maintenance of the proper level of this redox equivalent [37]. Some authors [31, 46] have observed a decrease in the activity of G6PD after chronic exposure to EtOH. The decrease in GR activity in the Pb-exposed rats could possibly be due to the interaction of Pb with -SH groups present at the active site of this enzyme, which in turn prevents the enzyme from participating in the reaction of GSSG reduction to GSH [13]. Jindal and Gill [17] have reported a decline in GR activity after exposure to Pb. Moreover, in some in vitro studies, G6PD was found to be inhibited by Pb [21, 49]. Also the formation of Pb-sulphydryl complexes was suggested as a plausible mechanism [22].

The co-exposure to Pb and EtOH caused a decrease in GR activity in the liver. ANOVA/MANOVA analysis revealed that the change in GR activity in the liver after the co-exposure to Pb and EtOH resulted from
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The increase in the renal activities of GPx and GR was demonstrated after the combined exposure to Pb and EtOH, similarly as after Pb exposure. ANOVA/MANOVA analysis revealed that the change in GPx activity resulted from an independent action of Pb or EtOH as well as from their interaction, due to which the renal GPx activity after the co-exposure to Pb and EtOH was at the same level as in the rats exposed separately to these xenobiotics. The increase in the renal GR activity in the rats co-exposed to Pb and EtOH resulted mainly from an independent action of Pb and was significantly higher in comparison to the rats exposed to these xenobiotics separately. The analysis of the percentage changes in the renal GR activity in the rats exposed to Pb and/or EtOH may suggest that the combined action of these xenobiotics has increased its potential.

It is possible that the animals treated with Pb alone and in conjunction with EtOH were affected by some degree of dehydration due to reduced fluid consumption in those rats, which might to some extent influence the results of this study. To eliminate the possibility of falsely altered results due to dehydration, the activities of GPx, GR and GST, like GSH and some other parameters determined in those animals, were expressed per mg of protein. However, when analyzing the results of the study, it has to be taken into account that the effect of both xenobiotics on the oxidative status, including the activities of GPx, GR and GST, may not only be caused by Pb and/or EtOH, but also by reduced fluid intake and dehydration.

On the basis of the results, it can be assumed that the decrease in the hepatic and renal GSH concentration found in the rats exposed to Pb and EtOH, both alone and in combination, may result from the changes in the activities of GPx, GR and GST in these organs. However, in order to explain completely the reasons for the decrease in GSH concentration in the liver and kidney due to the exposure to Pb and EtOH, especially in combination, it would be necessary to estimate their simultaneous influence on the activity of enzymes participating in GSH synthesis.

References:


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