THE ROLE OF GLUTATHIONE IN METABOLISM OF SELECTED DIMETHYLNAPHTHALENES IN RAT

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Abstract

Objectives: Methylnaphthalenes have been used extensively as chemical intermediates in organic synthesis, as solvents for pesticides, sulphur and various aromatic compounds. A wide use of methylnaphthalenes has contributed to their emission into the environment. The aim of the study was to explain the role of glutathione in metabolism of selected dimethylnaphthalenes in rat. **Materials and Methods:** The experiments were conducted on male rats of the strain outbred IMP:WIST. The animals were administered a single intraperitoneal dose (600 mg/kg body weight) of dimethylnaphthalenes (1,2-DMN; 1,3-DMN; 1,4-DMN) or dimethylnaphthalenes-[ring-U-3H]: (1,2-DMN-[3H]; 1,3-DMN-[3H]; 1,4-DMN-[3H]). The analysis was performed after 4, 8, 24, 48 h. The biochemical parameters were indicated: hepatic and pulmonary GSH, a-GST, SDH, GPX in blood, and adduct levels in the liver and lung. **Results:** The investigations demonstrated that a single intraperitoneal administration of dimethylnaphthalenes to rats at a dose of 600 mg/kg body weight caused a substantial depletion of reduced glutathione (GSH) level both in the liver and lung. The activity of a-glutathione S-transferases in serum of experimental animals exposed to dimethylnaphthalenes increased only after 1,2-DMN administration, the compound for which in earlier investigations the largest number of sulphur-containing metabolites was found in urine. To evidence that deep GSH depletion in analyzed organs has no oxidative nature, glutathione peroxidase activity in blood was determined. **Conclusions:** Lack of changes in glutathione peroxidase and sorbitol dehydrogenase activity for all the investigated compounds suggests that significantly deep GSH depletion in liver was not of oxidative nature and did not lead to necrotic changes in produced metabolites binding with GSH.

Key words:
Dimethylnaphthalenes, Glutathione, Metabolism, Rats

INTRODUCTION

Methylnaphthalenes have extensively been used as chemical intermediates in organic synthesis, solvents for pesticides, sulfur and various aromatic compounds. 2,6-Dimethylnaphthalene has been exploited as a precursor to 2,6-naphthalene-di-carboxylic acid, a useful monomer for polyesters [1]. 1,2-Dimethylnaphtalene in particular have been used as adjuncts in formulations for organophosphorus pesticide application and as solvents for fungicides [2]. A wide use of methylnaphthalenes has contributed to their emission into the environment. The presence of numerous derivatives of methylnaphthalenes in water and sea organisms has been described by a number of authors. Metabolism of dimethylnaphthalenes (DMN) was previously investigated in marine species [3,4]. Metabolism of 2,6-DMN was also examined in the liver microsomes isolated from untreated rats as well as from rats pretreated with a variety of agents known to alter the hepatic mixed function oxidase activity [5].

The aim of the study was to explain the role of glutathione (GSH) in metabolism of selected dimethylnaphthalenes in rat following a single intraperitoneal (i.p.) administration at a dose of 600 mg/kg body weight (b.w.) (about 50% LD_{50}). This work is a continuation of our earlier studies of

DMN metabolism in rat [6–8]. Our previous experiments revealed that DMN metabolism in rats encompasses ring hydroxylation, alkyl oxygenation and glutathione conjugation leading to methylthionaphthols. The amount of mercapturates (here rather sulphur-containing metabolites) excreted with urine differed significantly from 1.5 % for 1,4-DMN up to 35% for 1,2-DMN. In our opinion, the amount of sulfur-containing metabolites may reflect, at least in part, the amount of highly reactive metabolite formation, and obviously, the toxicity of parent compounds.

MATERIALS AND METHODS

Adult male outbred IMP:WIST (Rattus) of 180–220 g b.w. were obtained from the breeding colony of the Nofer Institute of Occupational Medicine in Łódź. The animals were supplied at least one week before the experiment and were fed a standard palletized Murigram diet (Agropol, Motycz, Poland) and had free access to water.

The animals were administered i.p. selected dimethylnaphthalenes (1,2-DMN; 1,3-DMN; 1,4-DMN) of analytical grade (Aldrich, England) or dimethylnaphthalenes-[ring–U-^{3}H] (1,2-DMN-[^{3}H]; 1,3-DMN-[^{3}H]; 1,4-DMN-[^{3}H]) purchased from the Department of Radiochemistry, Institute of Radiation Technique, Technical University of Łódź, with specific activity of about 400 kBq per animal, dissolved in olive oil at a single dose of 600 mg/kg b.w. (about 50% LD$_{50}$). The control group (n = 5) consisted of rats free from any injections. Rats were decapitated under light-ether narcosis at appropriate time intervals, and blood, serum, liver and lung were examined.

Concentrations of selected DMN-ring-U-^{3}H and its adducts with macromolecules were determined in liver and lung. For the quantification of DMN-ring-U-^{3}H and its metabolites, liver and lung samples were dissolved in 1N NaOH. The solution was acidified and mixed with scintillation fluid (Hydroluma, Baker, Germany). Radioactivity was counted in LKB-1209 rack beta liquid scintillation counter (with color correction program). The level of covalently bound adducts of DMN-ring-U-^{3}H was estimated as previously described by Jollow et al. [9]. The adducts were precipitated with trichloacetic acid (TCA) and the sediment was rinsed with methanol, n-heptane and ethyl ether. The sediment was subsequently dissolved in 1N NaOH and mixed with hydroluma.

Activity of α-glutathione S-transferases (α-GST) in serum was measured using the EIA Hepkit ™-Rt kit (Biotrin, Dublin, Ireland).The test procedure of this quantitative solid-phase enzyme immunoassay is based upon the sequential addition of sample, antibody-enzyme conjugate and substrate to microtiter cells coated with rat α-GST antibody. The resultant color intensity is proportional to the amount of l-GST in the sample. The assay range is 0–5000 μg/l. GST activity in serum was determined on a Beckman SYNCHON CX system.

Activity of glutathione peroxidase (GPX) in blood was measured using RANSEL kit (UV method – Randox, UK). GPX catalyzes the oxidation of glutathione by cumene hydroperoxide (substrate). In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP$^+$. The decrease in absorbance at 340 nm is measured. GPX concentration may be calculated from the following formula: IU/L of haemolysate = 8412 • DA 340 nm/minute and GPX activity expressed in IU/g Hb.

Activity of sorbitol dehydrogenase (SDH) in serum was determined spectrophotometrically using fructose as a substrate and NADH$_2$ as a coenzyme. Results were calculated using the molar extinction coefficient at 340 nm, according to the formula: IU (25°C) = DA/min • 645 umol min $^{-1}$ l^{-1}.

Glutathione leveling the liver and lung were determined colorimetrically using Ellman reagent – 5,5'-dithiobis-(2-nitrobenzoic acid) according to Sedlak and Lindsay [10]. Calibration was carried out parallel to each series, using reduced glutathione as a standard. GSH concentration was calculated from the regression equation and expressed per 1 g wet tissue.

Malondialdehyde (MDA) leveling the liver was determined with thiobarbituric acid as described by Mihara et al. [11]. The absorbance of the butanol phase containing aldehyde was measured at 532 nm and concentrations were calculated from the extinction coefficient (1.56 • 10^5/mol/cm) given by Casini et al. [12].

Statistical analysis

The statistical significance was calculated by one-way analysis of variance, using parametric tests (Snedecor and Scheffe) or non-parametric procedures (Kruskal-Wallis and Conover) for multiple comparisons. For comparisons of two groups Student's t-test or the Wilcoxon test were used. Statistical significance was set at the level of 5%.

RESULTS

A single i.p. administration of DMN isomers at a dose of 600 mg/kg b.w. caused a substantial depletion of GSH level both in the liver and lungs of the experimental animals (Figs. 1 and 2). It has resulted from our earlier studies on the DMN biotransformation in rat organism that thioderivative compounds are some of the main metabolites of the above mentioned compounds identified in urine of the experimental animals probably as the effect of conjugation with GSH.

Figure 3 presents the results of α-GST concentration in serum of the study and control animals. Statistically significant increase in α-GST activity was demonstrated only for one DMN isomer (1,2 DMN) already 8 h after administration, at the time when a maximum decrease in GSH level for this compound was also observed. This observation seems to be of importance due to the fact that 1,2-DMN is a compound for which the highest participation of sulphur-containing metabolites was demonstrated.
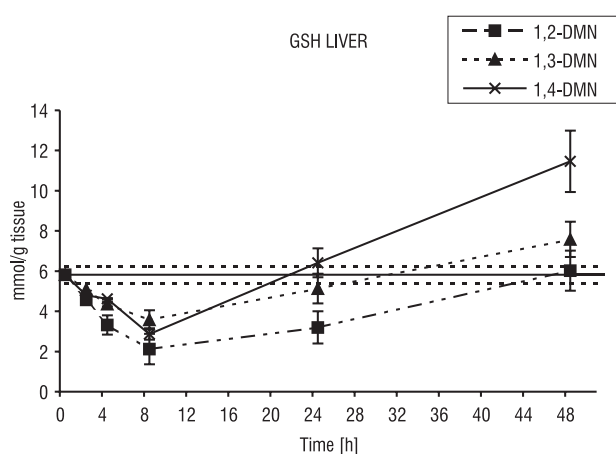

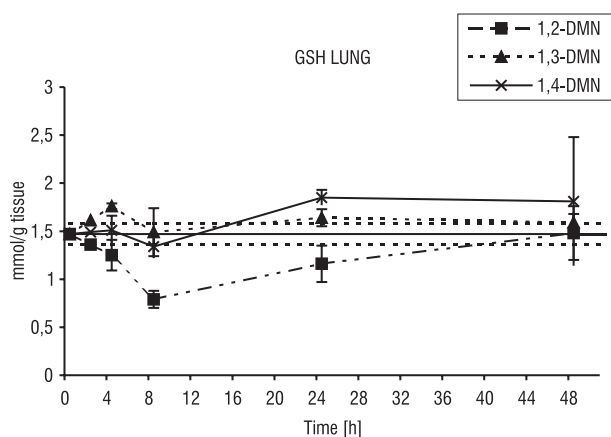**Fig. 2.** Pulmonary GSH ((μmol/g tissue) in rats after administration of dimethylnaphthalenes (i.p.) at a dose of 600 mg/kg b.w.

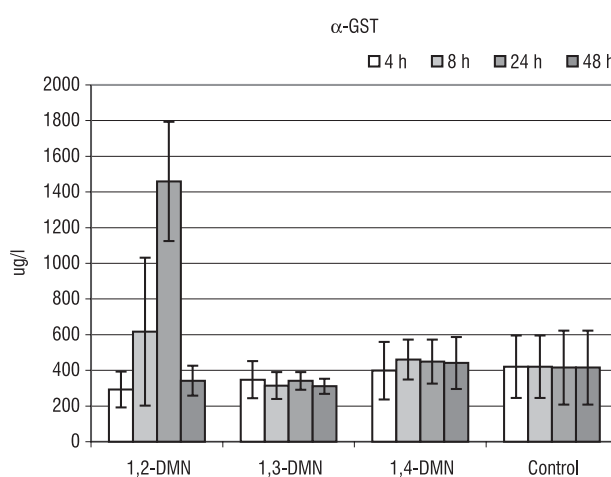**Fig. 3.** α-GST (μg/l) in serum after administration of dimethylnaphthalenes (i.p.) at a dose of 600 mg/kg b.w.

Figure 4 shows the results of glutathione peroxidase activity in rats examined after a single DMN administration as compared with the control group. This comparison does not indicate any significant differences in the activity of the enzyme in question.

Our experiment did not reveal statistically significant differences in the amount of malondialdehyde (final product of lipid peroxidation) in liver after administration of DMN isomers between the study group and controls.

The comparison of SDH activity in serum of study and control animals is given in Fig. 5. The results show lack of hepatotoxic activity after a single administration of the investigated compounds at a dose of 600 mg/kg b.w.

In another experiment, after administration of tritium labeled compounds to rats, the level of isotope permanently
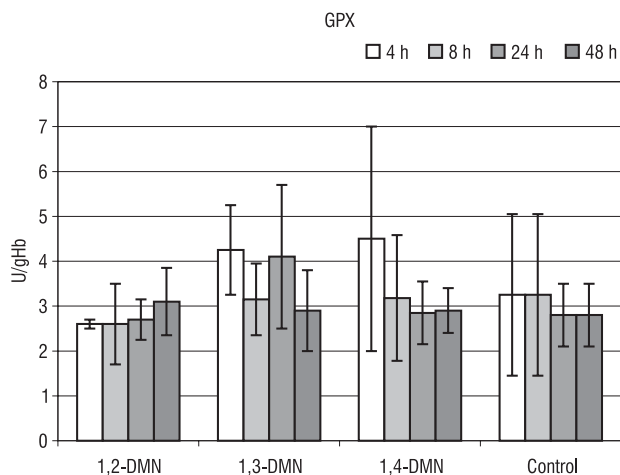

Fig. 1. Hepatic GSH (μmol/g tissue) in rats after administration of dimethylnaphthalenes (i.p.) at a dose of 600 mg/kg b.w.

GPX

□ 4 h   ■ 8 h   ■ 24 h   ■ 48 h

Fig. 4. GPX concentration (U/gHb) in blood after administration of dimethylnaphthalenes (i.p.) at a dose of 600 mg/kg b.w.
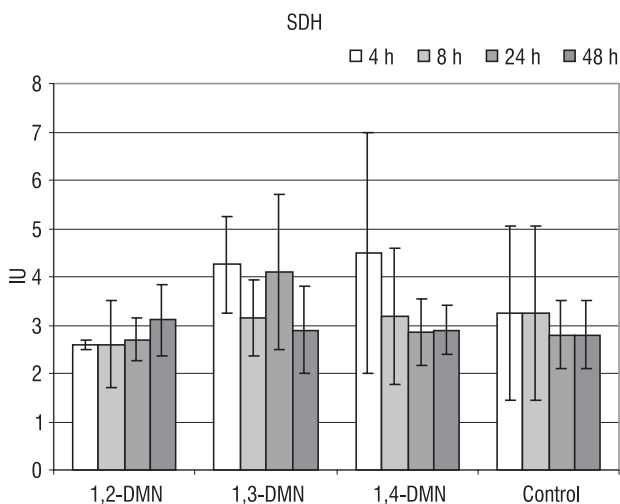
SDH

□ 4 h   ■ 8 h   ■ 24 h   ■ 48 h

Fig. 5. SDH (IU) in serum after administration of dimethylnaphthalenes (i.p.) at a dose of 600 mg/kg b.w.

LIVER   □ 4 h   ■ 8 h   ■ 24 h
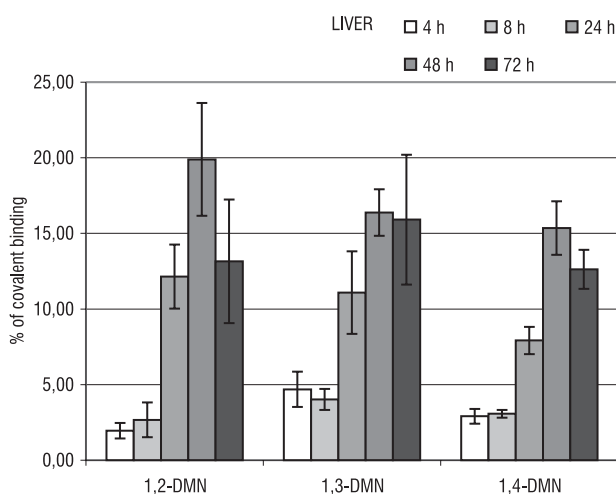   ■ 48 h   ■ 72 h

Fig. 6. Comparison of adduct levels permanently bound in liver after administration of dimethylnaphthalenes (i.p.) at a dose of 600 mg/kg b.w.

LUNG   □ 4 h   ■ 8 h   ■ 24 h
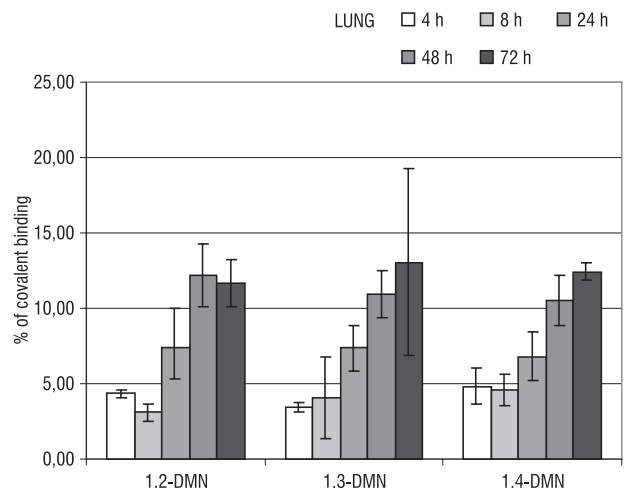   ■ 48 h   ■ 72 h

Fig. 7. Comparison of adduct levels permanently bound in lung after administration of dimethylnaphthalenes (i.p.) at a dose of 600 mg/kg b.w.

bound in liver and lungs was determined (Figs. 6 and 7). Also the level of adducts, permanently bound in liver and lungs was tested. The results of this study show that all the investigated compounds form a permanent adduct in liver and lungs 48-72 h after administration.

DISCUSSION

Conjugation to the nucleophilic thiol-group of the tripeptide glutathione is an important route of metabolism for a large number of lipophilic xenobiotics and endogenous compounds possessing an electrophilic center [13]. GSH-conjugation is also frequently referred to as the mercapturic acid pathway [14].

Besides GSH availability, glutathione S-transferase activity (mainly of isoform α) that catalyzes the majority of processes involving conjugation with GSH also affects the effectiveness and rate of this process. Glutathione S-transferases are major detoxication enzymes of phase II found in all eukaryotic organisms [15]. They are composed of a complex super-gene family that collectively metabolize a broad range of compounds including chemotherapeutic drugs, carcinogens and environmental pollutants [16,17]. The GSH transferases may be important in the DMN detoxication as is the case with naphthalene. Apart from epoxide hydrolysis, GST provides a major detoxication process for naphthalene epoxides [18,19]. The conjugation of xenobiotics with GSH is an essential part of xenobiotics' metabolic process. Apart from GSH availabil-

ity, GST activity mostly contributes to the efficiency of the metabolite-tripeptide binding process [20].

Naphthalene metabolized with depleted glutathione, or in the absence of sufficient glutathione, is bound covalently to tissue macromolecules. Correlation between bound metabolite levels and the intensity of lung injury suggests an association between reactive metabolites binding and toxicity. Reactive naphthalene metabolites are bound covalently to macromolecules in the lung, liver, and kidney, and this binding shows a pronounced glutathione threshold. Pretreatments that modify the level of covalently bound metabolites in the lung alter the toxicity in a parallel fashion (administration of naphthalene either via parenteral route or by inhalation). The survey of the literature data on the mechanism of toxic activity of naphthalene methyl derivatives (pneumotoxicity) shows that it is most probably associated with the formation of highly reactive metabolites which further on bind permanently in lungs and probably in the liver as well.

The study demonstrated that a single intraperitoneal DMN administration to rats at a dose of 600 mg/kg b.w. caused a substantial depletion of reduced glutathione level both in the liver and lung.

The results of the study show that α-GST activity in serum of experimental animals exposed to dimethylnaphthalenes increased only after 1,2-DMN administration. In earlier investigations of this compound, the largest number of sulphur-containing metabolites was found in urine.

The decrease in GSH concentration in the liver may lead to the increased lipid peroxidation in consequence of reperfusion after organs anoxia [21]. Lipid peroxidation is thought to cause accumulation of glutathione oxidized form, and the products formed in this process react with GSH, which results in further decrease in the GSH level and disturbance of the cellular oxidation-reduction system. Unfavourable effects of the decreased GSH concentration in cells exposed to oxidative stress seem to be conditioned by the decrease in two important detoxication reactions: reductions of peroxides and reactions of conjugation of aldehydes formed in the process of lipid peroxidation. In our study, the amount of MDA was the same in the exposed and control groups. Therefore, deep GSH depletion in rats after administration of the investigated compounds, was not correlated with the enhancement of the lipid peroxidation process, and in fact was mainly associated with the conjugation processes with compound metabolites under study.

In mammalian tissues, glutathione peroxidase catalyzes reduction reactions of H_2O_2 and organic peroxides by GSH. In our study, there were no significant differences in the activity of the investigated enzyme in whole blood in all exposed groups as compared to controls.

To check whether the decreased GSH level in the liver contributes to its damage leading to necrosis, sorbitol dehydrogenase was determined in serum of experimental and control animals. This revealed the lack of hepatotoxic activity after a single administration of the investigated compounds at a dose of 600 mg/kg b.w. No significant changes in this enzyme activity were found after administration of any of these compounds.

The level of adducts, permanently bound in the liver and lung was also tested. The results of this study show that all the investigated compounds form a permanent adduct in the liver and lung 48-72 h after administration. The formation of adducts with macromolecules depends on GSH availability in the liver and lung. Having considered the fact that the highest levels of covalent bonds do not agree with the time of maximal depletion of glutathione, further studies and explanations are needed to clarify their mechanism.

At this stage of the project it is rather difficult to offer an explanation for the delay in peak α-GST activity in relation to maximum depletion of GSH level in the liver and lung. The delayed manifestation of the peak concentrations of protein adducts in the tissue implies that GSH may be involved in the conjugation of protein thiol DMN. The latter compounds are to be determined in future studies that are expected to provide a clear-cut resolution to the problem.

CONCLUSIONS

■ Thioderived compounds are formed as a result of binding of GSH with the major dimethylnaphthalene metabolites.

■ A significant increase in glutathione S-transferase activity was found only for 1,2-DMN, the compound for which the largest proportion of thioderived compounds was observed in urine.

■ The lack of changes in glutathione peroxidase and sorbitol dehydrogenase activity for all the investigated compounds suggests that significantly deep GSH depletion in the liver was not of oxidative nature and did not lead to necrotic changes in produced metabolites binding with GSH.

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