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THE EFFECT OF SELECTED AROMATIC BROMINE DERIVATIVES ON THE ACTIVITY OF GLUTATHIONE PEROXIDASE AND TRANSFERASE

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Abstract
Objectives: Glutathione (GSH) is an important element of antioxidative barrier. Its biological function consists in eliminating oxygen free radicals. It also acts as a co-substrate in numerous enzymatic reactions catalyzed by glutathione peroxidase (GPx) and glutathione S-transferase (GST). In our study we attempted to assess the effect of hexabromobenzene (HBB) and its metabolites on the level of GSH and related enzymes, GPx and GST. **Materials and Methods:** The experiments were performed on female Wistar rats. The investigated compounds (HBB, 1,2,4,5-tetraBB, 1,2,4- and 1,3,5-triBB) were administered intragastrically in three different doses (HBB: 15, 75, and 375 mg/kg; 1,2,4,5-tetraBB and 1,2,4-triBB: 8, 40, and 200 mg/kg; 1,3,5-triBB: 12, 60, and 300 mg/kg) for 7, 14, 21 or 28 days. GSH level and activity of GST and GPx were determined in the obtained material. **Results:** The highest activity of GPx and GST was observed after a 7-fold administration of all investigated compounds. Prolonged time of exposure caused the return to the control values. **Conclusions:** The study revealed that repeated exposure to aromatic bromine derivatives increases GPx and GST activity only in the initial phase of the experiment.

Key words:
Polybromobenzenes, Rats, Glutathione, Peroxidase, Transferase

INTRODUCTION

Appearance of reactive forms of oxygen is a natural consequence of oxygen metabolism. Oxygen free radicals affect fatty acids in cellular membranes, initiating the process of peroxidation. They may also react with proteins, causing their oxidation and with deoxi- and rybonucleine acids, which is especially dangerous because of possible mutations [1].

Aerobic organisms create a number of defensive mechanisms in order to protect cells from the effects of active forms of oxygen. Glutathione peroxidase (GPx) is an important element of antioxidative barrier. This enzyme catalyzes the reduction of peroxides of unsaturated fat acids and hydrogen peroxide to less toxic alcohols and water. The reaction of hydrogen peroxide reduction is also catalyzed by another enzyme – catalase. However, this process seems less effective, since significantly higher concentrations of hydrogen peroxide are required to induce its activation compared with concentrations necessary for GPx activation [2]. Moreover, in contrast to other antioxidative enzymes, GPx acts within both hydrophobe and hydrophile areas [3]. GPx (currently known in at least

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5 forms) acts as oxidoreductases, showing high substrate specificity, where glutathione (GSH) provides electrones for these enzymes.

Glutathione belongs to antioxidants that constitute a secondary line of cellular defense from the effects of active oxygen forms [4]. Its biological function consists in eliminating oxygen free radicals ($^{\cdot}O_2^-$, $^1O_2$, $^{\cdot}OH$). It also acts as a co-substrate in numerous enzymatic reactions cathalyzed by GPx and glutathione S-transferase (GST). GSH also plays an important protective role by decreasing toxicity of many xenobiotics. Its binding to electrophile aromatic and aliphatic compounds results in the formation of conjugates, which are excreted from the cell. GSTs that show very high specificity in relation to GSH participate in the formation of conjugates [5].

Polybrominated aromatic compounds are used in many branches of industry as flame retardants. Production and usage of these compounds have significantly increased in recent years, which resulted in contamination of the environment with aromatic bromine derivatives [6]. These compounds as well as their metabolites can be found in river and sea residues, fish, molluscs and other sea creatures.

Hexabromobenzene (HBB) is one of the commonly used flame retardants. It belongs to so called additive flame retardants (incorporated into polymer), which facilitate its elimination and contamination of the environment. HBB, under the influence of different factors (UV radiation, high temperature), undergoes the process of debromination to penta-, tetra-, and tribromobenzenes [7,8].

In the present study we attempted to assess the effect of HBB and its metabolites, tetra- and tribromobenzenes, on the level of GSH and related enzymes, GPx and GST.

MATERIALS AND METHODS

The experiments were performed on female Wistar rats. The investigated compounds were suspended in sunflower oil and administrated intragastrically in 3 doses:

- heksabromobenzene (HBB) – 15, 75, 375 mg/kg b.w.;
- 1,2,4,5-tetrabromobenzene (1,2,4,5-tetraBB) – 8, 40, 200 mg/kg b.w.;
- 1,2,4-tribromobenzene (1,2,4-triBB) – 8, 40, 200 mg/kg b.w.;
- 1,3,5-tribromobenzene (1,3,5-triBB) – 12, 60, 300 mg/kg b.w.

Paralelly, in each experiment the measurements were carried out in the group of animals not exposed to any of the above mentioned compounds (pure controls). The animals were decapitated under light-ether narcosis by intracardiac puncture, then blood and liver were collected. Sections were performed 24 h following 7, 14, 21 or 28 days of exposure to the investigated compounds.

Glutathione level (liver), GST activity (liver and serum) and GPx activity (whole blood) were determined in the obtained material. All the experiments were performed with consent of the local Ethical Committee for Experimentation on Animals (No. Ł/BD/80).

Glutathione was determined by Sedlak and Lindsay's method with Ellman's reagent (5,5'-ditio-bis(2-nitrobenzoic)acid) [9]; GST activity (E.C.3.1.27) according to Asaoka and Takahashi, using o-dinitrobenzene as a substrate [10]; and GPx activity (E.C.1.11.1.9) with a ready Ransel test (RANDOX).

The obtained results were statistically analyzed with SYSTAT 5.30 program. The significance of differences for the selected parameters was determined with Tukey's test after checking homogeneity with Bartlett's test [11].

RESULTS

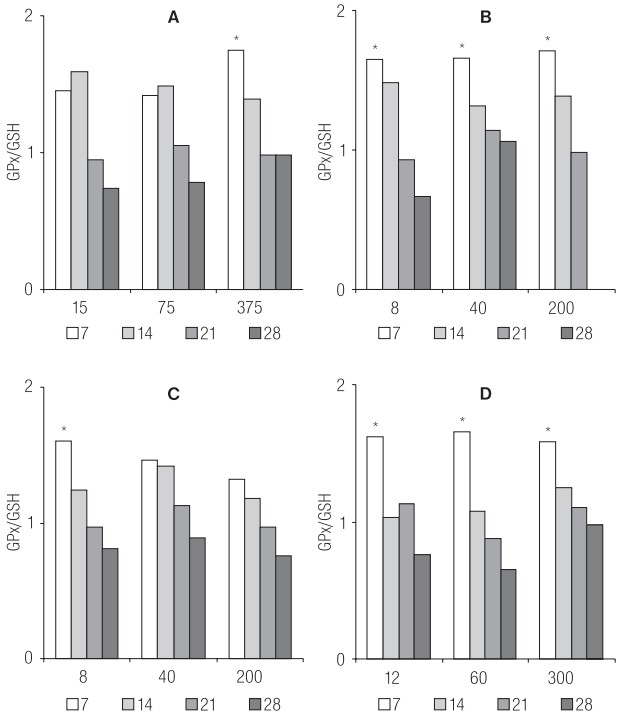
In the course of the experiments, GSH concentration as well as the activity of GPx and GST were assessed in the liver. To illustrate the changes induced by bromobenzenes, the following two indicators, calculated as the percentage of controls, were used:

1) the ratio of GPx activity to GSH concentration, and
2) the ratio of GST activity to GSH concentration.

Thus, the value of the indicator for controls was equal to 1 (100%/100%). The elevated value of the indicator suggested high activity of the enzyme at simultaneous low GSH concentration. The lower the value of the indicator, the lower the activity of the enzyme at elevated or unchanged GSH levels.

Changes in the GPx/GSH ratio after HBB administration to rats are illustrated in Fig. 1A. The increase in this parameter, reaching 140–175% (ratio 1.4–1.75), was detected after 7- and 14-fold administration of HBB (the changes were not dose-dependent). During the 3rd and 4th week of the experiment, the value of this parameter was significantly lower (max by 25%) as compared to controls.

Similar changes, but more pronounced, were noted after exposing rats to 1,2,4,5-tetra BB, which is illustrated in Fig 1B. The observed changes in the discussed parameter were less dependent on the dose than on the number of administrations, i.e. on the cumulative dose. Prolonged time of exposure to 1,2,4,5-tetraBB (administered in 3 doses) caused a successive decrease in the value of this parameter – most significant after the administration of the lowest dose (8 mg/kg). A dose of 200 mg/kg turned out to be so toxic that the animals did not survive 4 weeks of the experiment.



* Significant different as compared to controls, $\alpha = 0.05$.

Fig. 1. The GPx/GSH ratio in blood after repeated exposure (7–28 days) to HBB (A), 1,2,4,5-tetraBB (B), 1,2,4-triBB (C) and 1,3,5,-triBB (D) in 3 doses:
HBB – 15, 75, 375 mg/kg b.w;
1,2,4,5-tetra BB – 8, 40, 200 mg/kg b.w;
1,2,4-triBB – 8, 40, 200 mg/kg b.w.;
1,3,5-triBB – 12, 60, 300 mg/kg b.w.

Changes in the GPx/GSH ratio, correlated with the duration of the experiment, were also observed after repeated administration of 1,2,4-triBB in all doses. They were most significant after the lowest dose (8mg/kg): a 7-fold administration of this dose induced up to a 160% increase in this parameter (ratio 1.6), which declined to only 80% in the 4th week of the experiment (Fig. 1C).

The second isomer of tribromobenzene (1,3,5-triBB) caused even more pronounced changes in the GPx/GSH ratio. After administering the compound at a dose of 60 mg/kg, the value of this parameter increased up to approximately 170% (ratio 1.7) in the 1st week of the experiment, then it gradually decreased to 65% towards the end of the experiment. The slowest decrease in the ratio was observed after a dose of 200 mg/kg (Fig. 1D).

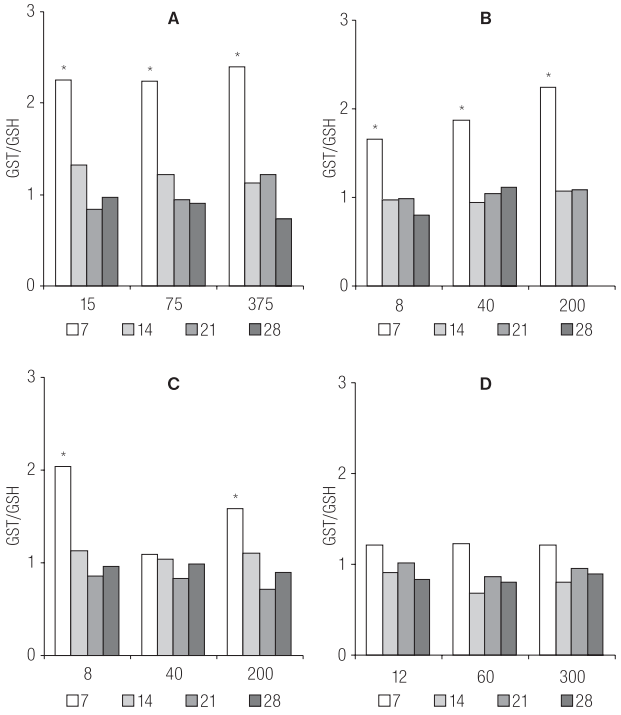
The other parameter (GST/GSH) to assess changes induced by repeated administration of bromobenzenes was obtained by calculating the ratio of GST activity in the liver to GSH concentration.

After all 3 doses, HBB initially caused an increase in this parameter (after 7 days of exposure) up to 225, 224, and 240% of control values, respectively (ratio, 2.2–2.4), followed by its slight decrease (most significant after a dose of 375 mg/kg) as illustrated in Fig. 2A.

Similar changes were observed after exposing rats to 1,2,4,5-tetraBB. A 7-fold administration of this compound induced the increase in the GST/GSH ratio, which was correlated with the dose and was equal to 1.6, 1.8, and 2.2, respectively (166%, 187% and 224% of control values). During the 2nd week of the experiment, the value of this parameter reached control values, whereas prolonged time of exposure did not result in any significant changes (Fig. 2B).

The increase in the GST/GSH ratio was also observed after 7-fold administration of 1,2,4-triBB at a dose of 8 mg/kg (ratio, 2) and 200 mg/kg (ratio, 1.6). At the remaining time points this parameter did not undergo any significant changes (Fig. 2C).

Figure 2D presents the changes in the assessed parameter induced by repeated administration of 1,3,5-triBB. The



* Significantly different as compared to controls, $\alpha = 0.05$.

Fig. 2. The GST/GSH ratio in rats after repeated exposure to HBB (A), 1,2,4,5-tetraBB (B),1,2,4-triBB (C) and 1,3,5-triBB (D) in 3 doses:
HBB 15, 75, 375 mg/kg b.w;
1,2,4,5-tetra BB 8, 40, 200 mg/kg b.w;
1,2,4-triBB 8, 40, 200 mg/kg b.w;
1,3,5-triBB 12, 60, 300 mg/kg b.w.

compound turned out to be a weaker inductor of changes in the activity of GST and GSH in the liver. This is illustrated by slight fluctuations in the GST/GSH ratio. This parameter reached the highest value after a 7-fold administration of all the doses (ratio, 1.2), while in the few weeks later it decreased reaching approximately 80% of control values (ratio, 0.8).

Changes in the GST/GSH ratio obtained in the serum are presented in Table 1. All examined compounds induced changes in this parameter of different extent, but of similar tendency. HBB administered in the lowest dose did not cause any changes. However, an increase in dose was accompanied by a decrease in this parameter. As regards 1,2,4,5-tetraBB and both tri-BBs, the highest values of this parameter were observed during the first and final phases of the experiment. The second and the third week of the experiment were characterized by the decreased value of the GST/GSH ratio.

Table 1. The GST/GSH ratio in rats after repeated exposure to bromobenzenes

Dose (mg/kg)	Duration of experiment (days)			
	7	14	21	28
		HBB		
15	1.04	1.06	1.01	1.09
75	0.96	1.05	0.96	1.03
375	0.86	1.01	1.06	0.86
		1,2,4,5-tetraBB		
8	1.20	0.94	0.87	1.05
40	0.99	0.91	0.98	1.41
200	1.07	0.91	1.12	–
		1,2,4-triBB		
8	1.00	0.67	0.75	0.86
40	0.92	0.61	0.93	0.94
200	0.81	0.57	0.76	0.80
		1,3,5-triBB		
12	0.80	0.61	0.81	0.90
60	0.92	0.66	0.64	0.81
300	0.91	0.69	0.99	1.12

DISCUSSION

The results of the experiments conducted in recent years still more often point to the fact that oxidative stress plays an important role in the pathogenesis of many diseases (e.g., diabetes, Parkinson's and Alzheimer's diseases) [12]. Reactive oxygen forms can also cause cellular impairment after exposure to xenobiotics. This issue evokes a growing interest in oxygen reactive forms as well as in enzymatic and non-enzymatic systems that prevent from their formation and activity. [13]. GSH, a compound that shows a surprising versatility, is one of the measures of the body's defense against oxidation-related impairments. The function of GSH, apart from eliminating free radicals, consists in decreasing the toxicity of numerous substances by binding to xenobiotic or its metabolite. Retaining an appropriate antioxidative potential, it acts as a defense mechanism against oxidative stress [14]. GSH participation of glutathione in detoxicating H_2O_2, organic peroxides and free radicals is possible due to GPx, an enzyme catalyzing the reaction of endogenous hydrogen peroxide reduction. The highest activity of GPx was observed in the liver, blood and

lungs, and the lowest in the brain and eye lens. [15]. An increase in GPx activity with a simultaneous decrease in the level of glutathione suggests that it is used by this enzyme [16]. However, it turned out that such direction of changes is not always the case. In experiments on rats, which were administered phosphoorganic insecticides, the increase in GPx activity was accompanied by unchanged glutathione concentration [17]. Similar tendency was detected after repeated exposure of rats to aromatic bromine derivatives. The observed changes were expressed as the ratio of GPx activity to GSH concentration (GPx/GSH). This parameter reached the highest value after a 7-fold administration of all (examined) compounds. Prolonged time of exposure and the resultant increase in the number of doses decreased the value of this parameter.

These changes were determined by changes in GPx activity (GSH concentration remained within control values) and their dependence was inversely proportional to the time of exposure (the larger the number of doses, the lower the value of this parameter). The lowest values of the GPx/GSH ratio were observed after 28 days of exposure to 1,2,4-triBB. This isomer caused a decrease in the parameter in all administered doses of approximately the same value.

Toxicokinetic tests of aromatic bromine derivatives proved that these compounds are excreted primarily with faces [18,19]. This route of elimination suggests that these compounds are transformed into metabolites, which are easily excreted with bile. GSH plays an important role in this process, as it is bound to an active form of xenobiotic (epoxide), forming mercapturic acid. Transition of nucleophile glutathione to electrophile molecules of xenobiotic is cathalyzed by enzymes belonging to the S-transferase group. The highest activity of these enzymes can be observed in the liver [20,21].

Previous experiments with use of aromatic bromine derivatives, conducted on rats and mice, proved that significant depletion of GSH results from a single exposure to these compounds [22–24]. The lowest GSH concentration in the liver of mice, reaching 15–20% of the control values, was detected after a single administration of bromobenzenes (BB) and dibromobenzenes (dBB). HBB, tetrabromobisphenol-A (TBBP-A) and 1,4-dBB had a considerably smaller effect. Exposure to these compounds decreased the level of GSH to approximately 60% of the control values. These experiments indicated that the low GSH level remained for no longer than 24 h after administration. Prolonged time of exposure probably triggers compensation mechanisms and results in the intensified glutathione synthesis.

A 28-day exposure of rats to HBB and 1,2,4,5-tetraBB decreased GSH level in the liver by 10% and 20%, respectively compared to the control group [25,26]. Our experiments seem to confirm this observation – prolonged exposure to aromatic bromine derivatives does not significantly affect GSH level in the liver. Repeated exposure of rats to HBB, 1,2,4,5-tetraBB, 1,2,3-triBB, and 1,3,5-triBB, however, induced considerable changes in the activity of the related enzymes, GPx and GST.

Probably GST, like GSH, undergoes changes in the activity following the same pattern. The highest GST activity was observed after a 7-fold administration of all investigated compounds. Prolonged time of exposure caused return to the control values. Because of its function, high activity of this enzyme should be accompanied by low GSH level. Our experiments do not confirm such a correlation. Perhaps the initial induction of GST activity results from the depletion of GSH pool in the liver, which takes place 24 h following the administration of bromine derivatives of benzene. Taking into consideration the induction of GST activity, the examined compounds can be put in the following order: HBB > 1,2,4,5-tetraBB > 1,2,4-triBB > 1,2,4-triBB > 1,3,5-triBB.

Experiments performed on mice to investigate acute toxicity of aromatic bromine derivatives showed that hepatotoxic effect depends on the number of bromine atoms as well as on their distribution in the molecule. It has been evidenced that the necrotic effect of these compounds decreases with the increasing number of bromine atoms [27]. Changing (reducing) the dose and type of exposure, from single to repeated, resulted in the changed direction of the toxic effect of bromine compounds in rats. Strong porphyrogenic effect of HBB was observed after repeated administrations, which was manifested by the increased

excretion of porphyrins in urine [26]. Similarly, the extent of changes in GPx and GST activity suggests that HBB, the compound with the largest number of bromine atoms in the molecule, is the substance exerting the strongest effect.

In conclusion, repeated exposure to aromatic bromine derivatives increases GPx and GST activity only in the initial phase of the experiment. The tendency of changes is similar – prolonged time of exposure results in gradual decrease in the activity of both enzymes and return to its control values.

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