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Effect of 4-methylpyrazole on antioxidant enzyme status and lipid peroxidation in the liver of rats after exposure to ethylene glycol and ethyl alcohol

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Abstract:
Background: The aim of the conducted studies was to evaluate the effect of 4-methylpyrazole, increasingly used in detoxifying treatments after ethylene glycol poisoning, on the activity of some antioxidant enzymes and lipid peroxidation formation in the liver of rats after experimental co-exposure to ethylene glycol and ethyl alcohol.
Methods: The trials were conducted on adult male Wistar rats. Ethylene glycol (EG) at the dose of 3.83 g/kg bw and ethyl alcohol (EA) at the dose of 1 g/kg bw were administered *po*, and 4-methylpyrazole (4-MP) at the dose of 0.01 g/kg bw was administered *ip*. Parameters of antioxidant balance were evaluated in hepatic cytosol, including the activity of the following enzymes: glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx) and lipid peroxidation level (TBARS).
Results: The results suggest that evaluation of the effects of administrated 4-MP after co-exposure to EG and EA in the liver revealed statistically significant changes on antioxidant enzyme system and malondialdehyde formation.
Conclusion: The changes in biomarkers activity indicate a greater production of free radicals which exceeds the capability of antioxidant system, appearing with oxidative stress in the group of animals treated by 4-MP combined with EG and EA.

Key words: 4-methylpyrazole, rat, liver, oxidative stress, ethylene glycol, ethyl alcohol

Abbreviations: 4-MP – 4-methylpyrazole, ADH – alcohol dehydrogenase, AlDH – aldehyde dehydrogenase, bw – body weight, CNS – central nervous system, EA – ethyl alcohol, EG – ethylene glycol, FDA – Food and Drug Administration, GPx – glutathione peroxidase, GR – glutathione reductase, GST – glutathione S-transferase, MDA – malondialdehyde, ROS – reactive oxygen species, TBA – thiobarbituric acid, TBARS – thiobarbituric acid reactive substances

Introduction

4-Methylpyrazole (4-MP) is a pyrazole derivative. It inhibits the activity of alcohol dehydrogenase (ADH) by binding the nitrogen atom of 4-MP with the zinc atom of the active enzyme. As an ADH inhibitor, it is used in the treatment of ethylene glycol (EG) and methanol poisoning. At much higher doses, 4-MP may inhibit EG and methanol oxidation in the system of cytochrome P450-dependent monooxygenases, thereby decreases the microsomal pathway of toxic formaldehyde production in EG biotransformation. In the liver, 4-MP is nearly completely metabolized, mainly into 4-carboxypyrazole and, to a lesser extent, into 4-hydroxymethylpyrazole. The average renal clearance of 4-MP is low and only 3% of the administered dose is excreted unchanged in the urine, indicating metabolism as the major route of elimination. The

generated metabolites are excreted with urine as glucuronides. With the involvement of cytochrome P450, 4-MP metabolism is autoinduced already within the first 30–40 h**.** 4-MP is eliminated from the body according to first-order or zero-order reaction kinetics, which depends on single and multiple doses of 4-MP [1, 3, 4, 16, 21, 24].

The main advantages of using 4-MP over ethanol in EG poisoning are: stronger ADH inhibition, no depressant effect on the central nervous system (CNS), slower elimination of 4-MP from the body, no need to monitor 4-MP concentrations. After 4-MP administration, ADH activity is inhibited and EG half-life in the serum is prolonged and the potency of processes generating toxic EG metabolites, e.g., calcium oxalate excreted with urine, is decreased. As well, in comparison to EA, 4-MP does not cause hypoglycemia 4–7, 9, 12, 15]. In 1986, 4-MP was accepted by the FDA (Food and Drug Administration) and it was indicated for use in adults in EG poisoning in 1997, and in methanol poisoning in 2000 [3, 4, 21, 24].

EG is a common cause of poisoning after it has been accidentally or intentionally consumed as a cheaper substitute for ethyl alcohol (EA). Both, EG and EA have a prooxidant effect and impair immune mechanisms of the system as they generate free radicals in the course of biotransformations. So far, studies on the EG toxic effect mechanism have been concentrated on the process of alcohol oxidation by the enzymatic system of dehydrogenases (alcohol ADH and aldehyde AlDH) into toxic metabolites: aldehyde and glycolic and glyoxylic acids. The emerging reports about the production of free radicals in the process of EG oxidation into formaldehyde mainly regard the action of cytochrome P450, and its isoform CYP2E1 in particular. The H_2O_2 created in the reaction catalyzed by CYP2E1 is crucial in the process of EG oxidation into aldehyde and may be a progenitor in the formation of reactive oxygen species (ROS). The created ROS may damage various intracellular structures and lead e.g., to DNA degeneration, lipid peroxidation or enzyme inactivation [11, 13, 18, 19].

EG poisoning is often complicated by simultaneous consumption of EA. Both in EG and EA poisoning, during the first biotransformation stages which may include cytochrome P-450 activity, the formation of lipid peroxidation products is enhanced and activity of enzymes participating in ROS removal increases. Another effect of poisoning with these compounds may be tissue hypoxia, which is related to the transformation of xanthine dehydrogenase into xanthine oxidase. Due to the smaller NAD^+/NADH ratio, xanthine dehydrogenase is transformed into xanthine oxidase. This leads to a greater generation of superoxide radical ion and hydrogen peroxide [2, 13, 17, 18].

To the best of our knowledge, there are no data regarding the influence of 4-MP on the generation of free radicals during EG and EA biotransformations. Therefore, we examined the influence of 4-MP on the antioxidant enzyme system and malondialdehyde (MDA) formation in the liver of rats in order to understand if there are toxicologically relevant amounts of ROS generated in severe poisoning with EG, alone or in combination with ethanol.

Materials and Methods

Studies were conducted at the Department of Toxicology, Poznan University of Medical Sciences, Poznań, Poland.

Chemicals

All chemicals from commercial sources were of the highest quality and were used without further purification. EG (99%) and 4-MP (99%) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA). Ethanol (99.8%) was obtained from POCh (Gliwice, Poland). All solutions were prepared immediately prior to their use.

Test animals and housing

The experiment was approved by Local Ethical Committee for Experiments on Animals (3/2004). The trials were conducted on adult male Wistar rats (260–300 g). Animals were housed in cages in controlled conditions of lighting 12/12 h, temperature 22 ± 2°C and humidity 55–60% with free access to food (Labofeed H) and water *ad libitum*. EG at the dose of 3.83 g/kg bw (1/2 LD_{50}) and EA at the dose of 1 g/kg bw were administered *per os*, and 4-MP at the dose of 0.01 g/kg bw was administered *ip*. Animals were divided into six subgroups and one control group of 5 rats in each of the 11 time points (385 animals were used for the present study). Xenobiotics were administered alone or simultaneously in the

same time, according to the following pattern: group I: 4-MP, group II: EG, group III: EA, group IV: 4-MP with EG, group V: EG and EA, group VI: EG + EA + 4-MP, group VII: controls. Rats were anesthetized with ketamine (Bioketan®) in the dose of 0.13 g/kg bw (*im*). Liver samples (8.9 ± 0.5 g) were collected after animal sacrifice at 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 36, and 48 h after xenobiotics had been administered. The study was conducted under a 48-h observation according to the path of EG metabolism.

Biochemical assays in hepatic cytosol

Microsomal fractions were isolated from the livers using the modified Dallner method [8]. Parameters of antioxidant balance were marked in hepatic cytosol, including the activity of the following enzymes: glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx) and lipid peroxidation level (TBARS). The activity of the antioxidant enzymes were presented in U/g, and TBARS concentration in μmol/g of cytosolic protein. The protein content of hepatic cytosol was estimated by the Lowry's method using bovine serum albumin as a standard [20]. For GPx marking, commercial diagnostic test was used, Ransel by Randox, respectively. GST was tested using the Mohandas method, taking 1-chloro-2,4-dinitrobenzene as the substrate [22]. GR was tested with the use of Glutatione Reductase Assay Kit II Cat. by Calbiochem. TBARS (as MDA) was marked in a reaction with thiobarbituric acid (TBA) [23].

Statistical analyses

Statistical analyses were performed with the use of the statistics program GraphPad InStat (3.06 version for Windows, GraphPad Software). Normal distribution of the data obtained was confirmed with the Kolmogorov-Smirnov test. ANOVA test was used to show the significant differences between experimental groups. If ANOVA detected significant differences ($p < 0.05$), Tukey test was used to compare the treatment groups *versus* the control group (*), EG group (#), EA group (°) and EG/EA group (^). The results were presented as the mean values ± standard deviation ($\bar{x} \pm SD$).

Results

The effect of 4-MP on the antioxidant enzyme system and MDA formation in the liver of rats exposed to experimental poisoning with EG, alone or in combination with EA, was studied. Biomarkers of oxidative stress were marked in hepatic cytosol, including the activity of the following enzymes: GST, GR, GPx and lipid peroxidation level (Tabs. 1–4).

Effects of EG, EA and 4-MP alone and their combinations on the antioxidant enzyme system and MDA formation

A single administration of EG significantly increased GR, GPx, GST activity and TBARS level by about 130, 40, 30 and 20% as compared with controls, respectively. Then, it was showed that a single administration of EA also caused an increase by about 90, 35, 120 and 40% as compared to control group, respectively. Similarly, it was also demonstrated during the experiment that a single administration of 4-MP caused an increase in GST (by about 120%), GR (by about 90%), GPx (by about 65%) activities and TBARS level (by about 15%), as compared to the control group, that lasted throughout the studied period (Tabs. 1–4).

In the rats co-exposed to EG and EA, significantly more pronounced alterations in the antioxidant enzyme system and MDA formation were observed during 48 h of the experiment in comparison with animals exposed to EG alone. At the same time, the GST activity increased by about 120% (Tab. 1). Furthermore, there were mild changes in TBARS level and GR activity, both up by about 30%, respectively (Tabs. 3 and 4). The GPx activity was lower in comparison with animals exposed to EG alone (by about 15%) (Tab. 2).

The rats treated simultaneously with EG and EA, in comparison with animals exposed to EA alone, demonstrated the changes in GR, GST, GPx activities and TBARS level, that were increased (Tabs. 1–4). However, selected measurements, in comparison to the group receiving ethanol, showed only slight changes, by about 15–40%.

In rats exposed to 4-MP in combination with EG, GST and GPx activities and TBARS level were higher than in rats exposed to EG alone (see Tabs. 1, 2, 4). Similarly, as compared to the group receiving EG, there were only 20–25% increases in GPx activity and TBARS level in groups which were adminis-

Tab. 1. Glutathione S-transferase (GST) activity (U/g) in hepatic cytosol in control group and after separate or simultaneous administration of ethylene glycol (3.83 g/kg bw) (EG), ethanol (1.0 g/kg bw) (EA) and 4-methylpyrazole (0.01 g/kg bw) (4-MP)

Time (h)	Control	EG	4-MP	EA	EG + EA	EG + 4-MP	EG + EA + 4-MP
0.5	359.4 ± 33.4	502.5 ± 62.1*	654.0 ± 58.3*	819.1 ± 38.9*	689.9 ± 24.5#°	970.1 ± 57.7#	197.2 ± 11.4#^
1	340.5 ± 68.4	496.1 ± 54.6*	666.2 ± 24.1*	837.7 ± 38.8*	984.9 ± 24.5#	974.7 ± 70.9#	147.3 ± 18.4#^
2	334.9 ± 24.8	492.9 ± 27.7*	859.8 ± 29.9*	808.4 ± 33.1*	863.1 ± 36.6#	818.0 ± 98.1#	138.0 ± 24.3#^
4	337.9 ± 13.5	453.5 ± 21.4*	769.8 ± 74.6*	963.4 ± 32.8*	1218.5 ± 17.4#°	707.9 ± 88.3#	138.7 ± 20.8#^
6	330.0 ± 6.7	441.1 ± 58.9*	771.3 ± 107.5*	1016.8 ± 26.7*	826.2 ± 27.6# °	714.9 ± 64.6#	120.0 ± 16.8#^
8	342.6 ± 22.6	398.1 ± 10.5	656.2 ± 97.2*	765.7 ± 44.9*	916.9 ± 31.1# °	638.1 ± 21.6	132.2 ± 18.5^
12	352.7 ± 40.3	386.0 ± 30.7	620.0 ± 75.3*	695.9 ± 27.9*	903.3 ± 32.6# °	664.5 ± 131.4	167.4 ± 14.0^
18	342.9 ± 18.7	320.2 ± 56.6	590.7 ± 71.9*	626.6 ± 32.4*	785.8 ± 39.5# °	562.1 ± 56.7	131.9 ± 10.3^
24	351.5 ± 36.2	335.3 ± 96.1	717.3 ± 28.1*	535.4 ± 34.9*	798.0 ± 40.3# °	683.3 ± 22.9	120.8 ± 9.6^
36	342.9 ± 34.2	307.1 ± 44.7	668.7 ± 64.3*	582.1 ± 21.0*	882.7 ± 16.4# °	695.9 ± 17.1	125.7 ± 6.8^
48	343.6 ± 12.4	420.5 ± 54.4*	661.0 ± 51.7*	459.7 ± 31.6*	732.9 ± 35.9# °	659.6 ± 50.9	161.0 ± 11.9^

$\bar{x}$ ± SD; significant difference: $p < 0.05$, * *vs.* control group, # *vs.* EG group, ° *vs.* EA group, ^ *vs.* EG + EA group

tered 4-MP in combination with EG. Also GR activity remained during the entire study on a level close to that in the group receiving EG. Our experiments showed that major changes were caused in GST activity (by about 80% of that in EG group).

The combined administration of EG, EA and 4-MP caused significant disorders in enzyme activity in the liver, often greater than those observed after the administration of EG or EA alone. The administration of 4-MP to animals exposed to EG and EA combined, resulted in a significant decreases in GST and GR activities (by about 60%) and a slight decrease in GPx and TBARS levels (by about 20–30%) in comparison with the EG group (see Tabs. 1–4).

Tab. 2. Glutathione peroxidase (GPx) activity (U/g) in hepatic cytosol in control group and after separate or simultaneous administration of ethylene glycol (3.83 g/kg bw) (EG), ethanol (1.0 g/kg bw) (EA) and 4-methylpyrazole (0.01 g/kg bw) (4-MP)

Time (h)	Control	EG	4-MP	EA	EG + EA	EG + 4-MP	EG + EA + 4-MP
0.5	579.2 ± 24.3	569.3 ± 40.3	1153.6 ± 85.2*	818.5 ± 83.1*	833.5 ± 43.2	1026.1 ± 130.2#	604.6 ± 14.2^
1	564.7 ± 29.7	849.5 ± 22.9*	1099.6 ± 102.3*	966.4 ± 35.4*	1172.5 ± 91.5#°	1159.4 ± 94.4#	720.5 ± 38.5^
2	575.2 ± 14.6	877.3 ± 134.9*	1298.6 ± 87.2*	711.1 ± 59.3*	840.3 ± 59.3	975.5 ± 91.7#	1090.1 ± 555.7^
4	588.4 ± 11.8	855.4 ± 39.4*	1170.0 ± 72.2*	1016.6 ± 99.4*	735.5 ± 98.1 °	1033.3 ± 58.7#	659.1 ± 135.9
6	604.7 ± 12.5	793.0 ± 27.6*	1086.4 ± 77.2*	823.9 ± 68.7*	774.4 ± 58.8 °	813.9 ± 79.4	560.5 ± 8.1^
8	599.1 ± 50.6	716.6 ± 93.2*	862.3 ± 46.3*	735.1 ± 89.2*	815.9 ± 61.8	727.1 ± 50.2	570.6 ± 76.4^
12	576.2 ± 63.6	748.5 ± 121.4*	796.3 ± 39.5*	631.9 ± 72.3	756.6 ± 58.3	842.8 ± 33.1	653.5 ± 76.7
18	616.6 ± 92.5	969.9 ± 56.3*	703.1 ± 68.6	665.0 ± 72.2	751.2 ± 47.4#	627.9 ± 64.5#	583.3 ± 55.3^
24	611.4 ± 31.1	949.3 ± 50.2*	857.4 ± 73.2*	559.4 ± 55.6	808.6 ± 75.2# °	707.1 ± 38.2#	549.9 ± 39.2^
36	582.0 ± 78.5	789.1 ± 153.3*	899.9 ± 24.9*	494.7 ± 67.4*	710.9 ± 66.9 °	879.7 ± 52.2	513.3 ± 60.7^
48	577.9 ± 89.7	750.2 ± 87.4*	763.3 ± 64.8*	487.1 ± 70.2	501.5 ± 48.9#	769.5 ± 71.2	482.9 ± 45.1

$\bar{x}$ ± SD; significant difference: $p < 0.05$, * *vs.* control group, # *vs.* EG group, ° *vs.* EA group, ^ *vs.* EG + EA group

Tab. 3. Glutathione reductase (GR) activity (U/g) in hepatic cytosol in control group and after separate or simultaneous administration of ethylene glycol (3.83 g/kg bw) (EG), ethanol (1.0 g/kg bw) (EA) and 4-methylpyrazole (0.01 g/kg bw) (4-MP)

Time (h)	Control	EG	4-MP	EA	EG + EA	EG + 4-MP	EG + EA + 4-MP
0.5	12.4 ± 1.2	28.8 ± 2.1*	25.4 ± 2.4*	22.5 ± 0.9*	24.0 ± 1.3#	29.5 ± 1.8	10.9 ± 0.4#^
1	11.5 ± 1.2	27.3 ± 2.0*	28.8 ± 1.1*	20.1 ± 0.6*	31.5 ± 1.4 °	33.0 ± 3.1#	10.6 ± 0.9#^
2	12.3 ± 0.8	29.8 ± 0.7*	30.2 ± 2.8*	24.9 ± 0.8*	41.0 ± 3.4#	28.4 ± 1.4	9.3 ± 1.1#^
4	12.8 ± 1.0	28.0 ± 1.8*	28.9 ± 3.2*	34.1 ± 3.3*	38.7 ± 1.1#	25.9 ± 1.4	10.2 ± 1.1#^
6	13.3 ± 0.9	29.2 ± 2.1*	27.7 ± 1.4*	25.4 ± 2.3*	32.6 ± 2.1 °	24.1 ± 0.9#	10.2 ± 0.9#^
8	13.8 ± 0.2	20.8 ± 0.8*	23.4 ± 1.7*	27.6 ± 1.1*	31.2 ± 2.6 #	23.5 ± 1.4	11.8 ± 1.5#^
12	13.4 ± 1.0	25.9 ± 2.3*	19.1 ± 2.0*	24.2 ± 1.6*	27.3 ± 2.2	20.5 ± 1.7#	10.6 ± 1.5#^
18	13.2 ± 0.5	33.8 ± 2.9*	18.4 ± 1.1*	20.4 ± 1.8*	27.7 ± 2.9# °	19.1 ± 1.8#	12.2 ± 0.4#^
24	13.2 ± 1.0	29.2 ± 2.3*	23.0 ± 1.4*	15.3 ± 1.3	29.9 ± 1.5 °	23.2 ± 2.7#	9.0 ± 0.4#^
36	10.8 ± 0.4	29.9 ± 3.0*	22.4 ± 1.6*	18.3 ± 0.6*	35.4 ± 1.3 ° #	23.6 ± 1.7#	10.1 ± 1.5#^
48	9.5 ± 0.4	25.2 ± 1.2*	20.4 ± 1.1*	20.9 ± 1.2*	30.1 ± 1.1# °	21.8 ± 1.6#	11.0 ± 1.0#^

$\bar{x}$ ± SD; significant difference: $p < 0.05$, * *vs.* control group, # *vs.* EG group, ° *vs.* EA group, ^ *vs.* EG + EA group

Tab. 4. TBARS concentration (μmol/g) in hepatic cytosol in control group and after separate or simultaneous administration of ethylene glycol (3.83 g/kg bw) (EG), ethanol (1.0 g/kg bw) (EA) and 4-methylpyrazole (0.01 g/kg bw) (4-MP)

Time (h)	Control	EG	4-MP	EA	EG + EA	EG + 4-MP	EG + EA + 4-MP
0.5	10.4 ± 0.4	12.3 ± 0.4*	8.2 ± 0.5*	12.8 ± 0.7*	9.4 ± 0.4# °	8.7 ± 0.3#	10.5 ± 1.4#
1	8.5 ± 0.4	9.4 ± 0.3	7.4 ± 0.5	12.4 ± 0.8*	12.1 ± 0.4#	8.5 ± 0.7	8.5 ± 0.6^
2	9.4 ± 0.1	11.2 ± 0.4*	7.7 ± 0.3	14.1 ± 0.7*	11.9 ± 0.5 °	9.5 ± 0.7#	8.1 ± 0.2#^
4	8.6 ± 0.4	10.2 ± 0.7	8.0 ± 0.7	15.5 ± 0.4*	17.9 ± 0.7# °	12.2 ± 0.4#	6.8 ± 0.2#^
6	10.2 ± 0.3	10.6 ± 0.7	10.6 ± 0.6	13.6 ± 0.3*	13.4 ± 0.9#	11.0 ± 0.8	7.9 ± 0.9#^
8	10.1 ± 0.6	8.6 ± 0.4*	11.4 ± 1.0*	11.7 ± 0.7*	12.4 ± 1.1#	11.2 ± 0.8#	8.2 ± 0.9^
12	10.1 ± 0.3	9.9 ± 0.5	10.3 ± 0.6	12.7 ± 0.4*	12.8 ± 0.6#	11.4 ± 0.8#	8.4 ± 0.6#^
18	9.6 ± 0.3	14.4 ± 0.6*	12.4 ± 0.6*	12.4 ± 0.5*	10.1 ± 0.5# °	12.7 ± 0.9#	9.3 ± 0.6#
24	8.0 ± 0.4	10.8 ± 0.6*	11.2 ± 1.0*	14.0 ± 0.9*	9.0 ± 0.4# °	11.1 ± 0.7	9.8 ± 0.8
36	9.3 ± 0.2	10.2 ± 0.5	9.4 ± 0.8	12.9 ± 0.8*	10.3 ± 0.3 °	8.5 ± 0.6#	12.0 ± 1.0#^
48	10.8 ± 0.7	6.9 ± 0.3*	7.3 ± 0.5*	11.8 ± 0.8*	11.2 ± 0.7#	8.7 ± 0.6#	8.9 ± 0.3#^

$\bar{x}$ ± SD; significant difference: $p < 0.05$, * *vs.* control group, # *vs.* EG group, ° *vs.* EA group, ^ *vs.* EG + EA group

Discussion

The toxic effects of ethylene glycol and ethanol are related not only to the production of toxic metabolites, but also to the significant influence of both alcohols on antioxidant balance of the body. Disorders in the balance between the amount of produced free radicals and their neutralization cause oxidative stress. Toxic EG metabolites created by the action of CYP2E1 diminish the antioxidant potential of cells; subsequently ROS, mostly hydrogen peroxide, are released. A coexistent intoxication with EA causes not only the activation of ADH/AlDH system, but also a decrease in NAD+/NADH ratio and a greater conversion of xanthine dehydrogenase into xanthine oxidase, during which ROS, hydroxyethyl radicals and ethoxyl radicals, are also generated [2, 11–13, 17–19].

The treatment of EG poisoning is based, among other things, on inhibiting the oxidative transformations leading to the generation of toxic metabolites by using EA or 4-MP. The effect of both compounds is based on the competitive inhibition of EG transformation into glycolaldehyde. However, they differ from each other in affinity to ADH and in pharmacokinetics. 4-MP is also less toxic than EA [1, 7, 12, 15].

In the present study, the interactive effects of combined exposure to EG, EA and 4-MP on the antioxidant enzyme system and malondialdehyde formation were assessed. We demonstrated the presence of antioxidant balance disorders in hepatic cytosol of rats after single and combined exposure to 4-MP, EA and EG. In this study, it was demonstrated that administration of each of three: EG, EA and 4-MP led to disruption in the antioxidant enzyme system and MDA formation in rats. These changes were evidenced by increases in GR, GST, GPx activities and TBARS level in the rats exposed to EG, EA and 4-MP alone and in decreases in activities of enzymes and TBARS level in the rats exposed simultaneously to EG, EA and 4-MP. The increases in activities of antioxidant enzymes and TBARS level in rats exposed separately to each of these three agents may indicate that antioxidant reserves were activated due to greater production of ROS during their biotransformation pathway. The most profound changes caused EG in GR activity (by about 130% *vs*. controls) and EA in GST activity (by about 120% *vs*. controls). Similarly, 4-MP caused the great changes in GR and GST activities (by 100%).

To sum up these results, it should be stated that in the course of metabolism of alcohols, including EA and EG, ROS are generated. This leads to the development of oxidative stress and disturbance in the homeostasis of the system. It proves that EG and EA administration leads to an increase in stationary concentrations of ROS. 4-MP also induce an increase of ROS production and activities of GR and GST. Subsequently, disorders in the balance between the amount of produced free radicals and their neutralization develop. These conclusions corroborate in part the reports of other authors [2, 6, 10, 12].

The increases in the activities of antioxidant enzymes and TBARS level in rats co-exposed to EG and EA showed significant changes, especially in GST and GR activities. The results of the current study demonstrated that EG and EA co-administration, in comparison with animals exposed to EG alone, leads to the development of oxidative stress and causes much higher increase in the activity of GST than EG and EA alone. The other markers in hepatic cytosol of the rats exposed simultaneously to EG and EA were also changed, but less significantly than the activity of GST or GR.

Following the combined exposure to EG, 4-MP and EA the activities of antioxidant enzymes were much lower than in the group exposed to EG only. This may indicate a relevant role of EA and 4-MP in aggravating of oxidative stress in rat liver and, in consequence, faster depletion of antioxidant reserves due to their interaction with ROS that are generated during both EG and EA biotransformation. 4-MP also seems to block markedly the effects of the combined administration of EG and EA on the antioxidant enzyme system and MDA formation, while at the same time not to affect or even increase the effect of EG alone.

Similarly, Jurczyk et al. showed a decreased activity of cellular antioxidants in red blood cells in rats in the course of acute EA poisoning, during which large amounts of ROS are produced [17]. Data presented by Dudka, regarding oxidation disorders in the rat brain due to EA and 4-MP administration in the experimental methanol poisoning, indicate a significant effect of both alcohols on the potential of antioxidants (TAS) and detrimental interactions of methanol with ethanol [10]. The observed decreased activities of GPx, GST, GR and TBARS level in rats that were co-exposed to EG, EA and 4-MP may indicate that these enzymes are gradually used in response to the overproduction of ROS.

Conclusion

4-MP inhibits EG biotransformation but it does not seem to have antioxidant effects, which is proved by observed antioxidant balance disorders in the group of animals that simultaneously received EG, 4-MP and ethanol. The results indicate that interactions between xenobiotics during the biotransformation stage have a significant effect on the antioxidant balance in the rat liver.

Additionally, the results in the group of animals that received all xenobiotics, EG, 4-MP and EA, may suggest that the total effect of these compounds induce the oxidative stress in the hepatic cytosol, which was shown as the changes of antioxidant enzyme activities and lipid peroxidation. In response to this, antioxidant defense mechanisms were induced.

In the present study, the changes in biomarkers activities indicate a greater production of free radicals appearing with oxidative stress in the group of animals that received 4-MP combined with EG and EA. It may be a result of gradual reduction of antioxidant enzymes status and lipid peroxidation in response to the overproduction of ROS. In fact, the timing of 4-MP administration could also have an important influence on the results. Furthermore, some other measurement of oxidative stress, e.g., GSH/GSSH levels, could be more appropriate and profitable for the successive experiments.

The obtained results suggest that in this experimental model, the evaluation of the effects of administered 4-MP after co-exposure to EG and EA revealed statistically significant changes in antioxidant enzyme system and MDA formation in the rat liver.

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