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ROLE OF ESTRADIOL IN REDUCING ETHANOL TOXICITY

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Estrogens play multifunctional role in the body. The last decades research were focused on its free radicals processes participation (1–3). The antioxidative, but also the prooxidative properties of estrogens were the subject of interest (1). The aim of our research is to investigate whether the estrogens participate in detoxification processes in exposure to xenobiotics, particularly free radicals pathway. The presented study examines the influence of ethanol and estradiol on natural enzymatic antioxidative barrier of superoxide dismutase (SOD) and glutathione peroxidase (GPx). In order to evaluate the interaction and the role of estradiol in detoxification the joint effect is examined. It could be helpful in the evaluation of women sensitivity to xenobiotics, especially in post-menopausal period, and also in finding the differences of sex-dependent reaction. Our previous research showed that 17-β-estradiol (E2) inhibits lipids peroxidation caused by the fluoride and that the mechanism is connected to higher extent with thiol groups protection than hydroxyl radical scavenger (4). There are only very few reports on estrogen-xenobiotics interaction. One is about harmful effect of smoking on hormonal replacement therapy (HRT) (5, 6). The second reports that alcoholic drink increases the level of estrogens in human blood (7).

EXPERIMENTAL

The material was the rest fresh blood received from Academic Hospital. The research was performed *in vitro* on human blood erythrocytes isolated from blood taken on EDTA (SOD) or blood taken on heparine (GPx). The SOD activity was measured with RANSOD test (Randox Laboratories). Blood samples were centrifuged for 10 min at 3000 rpm, plasma was rejected and erythrocytes were washed with 0.9% sodium chloride making dilutions 4 times. The suspension of 10% blood corpuscules in PBS was prepared. Assay principle was based on the ability of SOD to accelerate the dismutation of the superoxide radical. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The superoxide dismutase activity is measured by the degree of inhibition of this reaction. One unit of SOD causes a 50% inhibition. Absorbance was measured on Spectrophotometer U-2900 at 37°C at the wavelength λ = 505 nm. The GPx level was evaluated by commercial test RANSEL (Randox Laboratories) with method described by Paglia and Valentine (8).

The ethanol (96% pure p.a., Chempur) in concentrations of 0.4, 0.5, 0.8, 2.0, 2.4 and 4.0 mg/mL and 17-β-estradiol (FW = 272 g/mol, Sigma-Aldrich) in the concentrations of 0.75 nM, 1.0, 5.0 and 10.0 μM were used. The results were evaluated by statistical analysis (program Statistica 8.0) with ANOVA or Student's *t*-test.

RESULTS AND DISCUSSION

It was observed that ethanol in conc. 0.5 and 2.0 mg/mL significantly ($p < 0.05$) increased the activity of SOD in erythrocytes in comparison to the control (Fig. 1). The effect of ethanol acute intoxication is quite different than one observed in long-lasting exposure, specially the addiction (decrease) (9, 10). 17-β-Estradiol at physiological level (0.75 nM) didn't influence on SOD activity, but at higher

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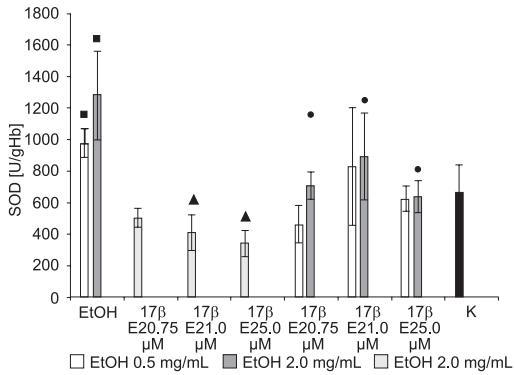

Figure 1. The influence of ethanol, estradiol and ethanol with estradiol on SOD activity. ■ $p < 0.05$ compared to control, ▲ $p < 0.001$ compared to control, ● $p < 0.05$ compared to EtOH; K = control

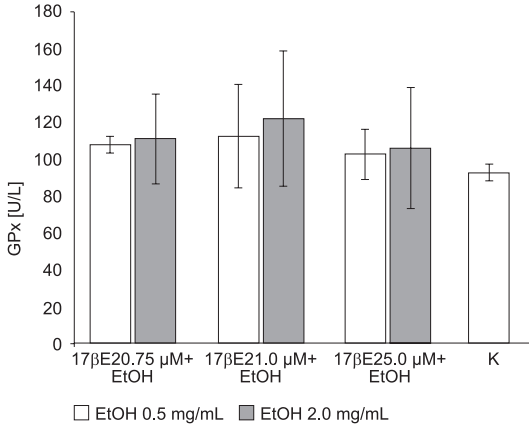

Figure 2. The influence of ethanol with estradiol on GPx activity. K = control

Table1. Influence of ethanol (EtOH) and estradiol (17β-E2) on GPx activity.

EtOH	GPx [U/L]	p value
EtOH 0.4 mg/mL	81.538 ± 3.338	< 0.01
EtOH 0.8 mg/mL	114.26 ± 3.044	< 0.00001
EtOH 2.4 mg/mL	97.59 ± 3.288	< 0.0001
EtOH 4.0 mg/mL	89.916 ± 3.991	< 0.001
$Control_{EtOH}$	73.942 ± 1.625	
17β-E2		
17β-E2 0.75 nM	56.9 ± 9.7	< 0.001
17β-E2 1.0 μM	41.9 ± 5.32	< 0.001
17β-E2 5.0 μM	21 ± 5.6	< 0.001
$Control_{17β-E2}$	92.8 ± 8.16	

conc. (1.0 and 5.0 μM) significantly ($p < 0.001$) decreased enzyme activity (Fig. 1). The joint effect of ethanol and estradiol on SOD (every concentration of ethanol with every concentration of estradiol) didn't differ from the control (Fig. 1). The comparison of joint effect with single ethanol action points on estradiol inhibition of oxidative stress caused by ethanol, because ethanol itself increased SOD activity. The conclusion is confirmed by statistical analysis ($p < 0.05$) (Fig. 1).

The research on ethanol influence on GPx activity showed the significant increase ($p < 0.05$) in comparison to the control (Tab. 1). Estradiol in every examined concentration decreased the GPx activity in erythrocytes (Tab. 1). The joint effect didn't show the interaction (Fig. 2). The action of ethanol with every concentration of estradiol was comparable with the control. Because ethanol itself increased the GPx activity, the inhibiting effect of estradiol on oxidative stress caused by ethanol is noted. It is known that oxidative stress stimulate the activity of natural defense system SOD and GPx. The lack of stimulation could be the result of oxidative stress inhibition, however, the direct effect of xenobiotics on enzyme can't be excluded. In summary, it seems that estradiol partly reduces oxidative stress caused by ethanol in the influence on natural enzymatic barrier activity.

There are many reports discussing how ethanol induce oxidative stress in the body (11–13). The higher level of lipids peroxidation and lower concentration of natural antioxidants were noted in alcoholics [9, 10]. The SOD activity measured in 1 h of acute intoxication of rats with ethanol (2.4 and

6 g/kg b.w.) wasn't changed in lung and kidney but decreased in liver (12). The reports confirmed tissue-specific changes of activity and its dependence on the type of intoxication (acute or long-lasting). The majority showed the SOD decrease in long-term ethanol intoxication. Such decrease was noted in rats erytrocytes in 8 and 12 weeks intoxication with 1/10 LD_{50} (13). The are no reports how SOD activity is changed in erythrocytes in acute intoxication. Our investigation showed that ethanol in conc. 0.4–4.0 mg/mL increases SOD activity in human erythrocytes (Fig.1). It correlates with reports on SOD activity in serum. In serum of rats treated intragastrically with 50% ethanol in a dose of 5 mL/kg b.w., the increase of SOD activity was noted in 2 h, maximum in 12 h and a decrease after 24 h (14). The other research (15) also reports the increase of SOD activity in plasma of rats in 4 and 6 h after acute intoxication with doses 2, 4 and 6 g/kg b.w., however, the decrease in 1 h. It seems that direct answer for oxidative stress is the mobilization of natural barrier, what means an increase of SOD activity. The very interesting research on SOD activity in brain (16) points on the role of isoenzymatic form. In the acute ethanol intoxication, the decrease of CuZn-SOD was noted in cytosolic and microsomal fraction of brain but the decrease of Mn-SOD in mitochondria. The research on alcohol-addicted people showed even 85% decrease of SOD activity in serum (28 men). The low SOD activity was observed still in 14 days of abstinence (9).

The research of estradiol influence on SOD showed the decrease of its activity in brain, heart, liver and vagina of female rats in aging process (17, 18). The increase after hormonal replacement therapy (estradiol in dose 0.1 μg/g b.w. (17) and estradiol in dose 40 μg/kg/day (18)) was noted. The various results were obtained for SOD isoenzymatic form (19). The treatment of ovariectomized rats with single dose of 5 μg of estradiol 17β-benzoate caused a decrease of mitochondrial Mn-SOD in the brain but no changes in cytosolic CuZn-SOD. The examination of 80 Polish women didn't show the differences in SOD activity in erythrocytes after menopause (2). The other research (Brasilian women) reported the decrease of SOD at postmenopausal period (3, 20). Our study showed inhibiting effect of estradiol in conc. of 1.0 and 5.0 μM on SOD in erythrocytes (Fig. 1). This inhibition is also observed in the presence of ethanol (Fig. 1). Ethanol itself stimulate SOD activity, but in the presence of estradiol it didn't. The combined effect is comparable to the control and no interactions were noted (Fig. 1).

The influence of ethanol on GPx activity depends on kind of intoxication (acute or long-term). In the first case, changes were organ-specific, e.g., acute intoxication of rat at doses 2 ,4 and 6 g/kg b.w. induced an increase of GPx activity in lungs, a decrease in kidneys and no significant changes in the liver (12). In other studies, there was no influence on GPx activity in plasma of rats in 1 hour after intoxication with ethanol 2, 4 and 6 g/kg b.w., what could suggest the low level of hydrogen peroxide and lipid peroxide (15). However, there was an increase of GPx activity in 2 hours after stomach tube administration with 50% solution of ethanol in a dose of 5 mL/kg b.w. A decrease of GPx after 12–24 hours of intoxication could be related to low availability of glutathione, used by ethanol (14).

The significant ($p < 0,02$) decrease of GPx activity in liver and kidneys of Fisher-rats was the result of chronic ingestion, with 20% solution of ethanol at a dose of 2 g/kg b.w. during 6.5 weeks. Significant changes were not observed in lungs and testes of the animals (21). Investigation on patients with alcohol dependence demonstrated that activity level of GPx in serum were significantly ($p < 0.05$) lower in the alcoholic patients than in control subjects (9).

The researches about the influence of estradiol on GPx activity are very rare. The study with 80 postmenopausal women (2) found that erythrocyte GPx activities were lower than in the control group. The GPx activity diminished with aging processes. The hormonal replacement therapy (HRT) caused an increase of GPx (estrogen and progesterone treatment) (2). Different results were obtained in the research on 48 Brasilian women (3). The level of enzyme activity (GPx) was measured in two groups of these women: before (control group) and after menopause. Post-menopausal women were categorized into group with or without HRT treatment. The results showed no significant changes in the postmenopausal group and HRT treated.

In summary, the influence of ethanol on SOD and GPx activity is tissue-specific and depends on the type of ingestion. The increase of SOD and GPx activity in erythrocytes caused by ethanol is partly reduces with estradiol.

CONCLUSIONS

Ethanol in all investigated concentrations increased SOD and GPx activities in erythrocytes.

Estradiol in physiological concentration (0,75 nM) didn't show influence on activity of SOD and GPx, but in doses 1.0 and 5.0 μM decreased SOD as well as GPx.

Combined effect of ethanol and estradiol didn't show the interaction but the inhibiting effect of estradiol on oxidative stress caused by ethanol.

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