Effect of Perftoran on Free Radical Homeostasis during Alcohol Withdrawal Syndrome

D. A. Miskevich, N. E. Petushok, P. A. Gerasimchik*, and A. N. Borodinskii

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 143, No. 1, pp. 49-51, January, 2007
Original article submitted May 31, 2006

We studied the effects of Perftoran on free radical processes in the liver of rats with withdrawal syndrome after chronic alcohol intoxication. Administration of Perftoran in the period of ethanol withdrawal eliminated the signs of oxidative stress and prevented elevation of enzyme activities in the blood, which reflects improvement of structural and functional characteristics of liver cell membranes.

Key Words: *Perftoran; free radicals; antioxidant system; oxidative stress; ethanol*

The increase in alcohol consumption in the world necessitates the search for new drugs for effective correction of pathochemical changes associated with alcohol abuse.

Perfluorocarbons, in particular Perftoran (PF), are characterized by a wide range of properties. They perform gas transport function [1] and produce antioxidant [2], immunomodulatory [9], and membrane protective effects. This prompted us to study the influence of PF in alcohol-induced damage to internal organs.

Here we studied the effect of PF on metabolic status of the organism during the withdrawal period after chronic alcohol intoxication (CAI).

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 160-180 g. The animals were maintained in a vivarium and fed a standard diet. The animals were divided into 4 groups (8 rats per group). Experimental animals received 25% ethanol in a narcotic dose of 3.5 g/kg 2 times a day for 42 days through a tube. Control animals received an equivalent volume of distilled water instead of ethanol. Rats of experimental group 1 were decapitated 1 day after ethanol withdrawal. On days 3 and 6 after ethanol withdrawal, PF in a dose of 1 ml/100 g was infused over 1.5-2.0 min to group 3 rats via the femoral vein under calipsol anesthesia (20 mg). Group 2 rats received an equivalent volume of NaCl (0.95%). Rats of groups 2 and 3 were decapitated after 12-h food deprivation on day 7 after ethanol withdrawal.

The liver and blood plasma were analyzed. Activities of superoxide dismutase (SOD) [6], catalase [3], glutathione peroxidase [4], and glutathione reductase and contents of reduced glutathione and thiobarbituric acid-reactive substances (TBA-reactive substances) [10] were measured in liver samples after centrifugation at 12,000 rpm. Activities of alanine transaminase (ALT), aspartate transaminase (AST), and γ-glutamyltranspeptidase (GGTP) and content of nitrites (NOx) [9] were measured in blood plasma.

A fragment of the liver was washed with ice-cold 0.9% NaCl and frozen in liquid nitrogen. These samples were homogenized at 12,000 rpm. Nitrite/nitrate content (NOx) was measured using Griess reagent [9].

The results were analyzed with Microcal Origin 6.0 software.

Laboratory for Regulation of Metabolism, Institute of Biochemistry, National Academy of Sciences of Belarus; *Grodno Regional Clinical Hospital. ***Address for correspondence:*** mda1977@rambler.ru. D. A. Miskevich

RESULTS

Signs of oxidative stress (imbalance in SOD and catalase activities and activation of peroxidation processes) were found in animals after CAI, which can be explained by increased H_2O_2 concentration. This assumption is indirectly confirmed by catalase activation (Fig. 1). SOD is not the source of catalase substrate H_2O_2, because SOD activity remained unchanged under these conditions (Table 1). Activation of nonenzymatic production of H_2O_2 induced by CAI seems most likely. Cytosolic H_2O_2 is utilized by glutathione peroxidase. However, we did not reveal a significant increase in glutathione peroxidase activity (Table 1). Hence, the increase in cytoplasmic H_2O_2 concentration can be anticipated.

The imbalance of antioxidant enzymes contributed to activation of peroxidation processes. The concentration of TBA-reactive substances increased by more than 1.5 times (Fig. 1). Changed permeability of the blood-tissue barriers in the liver manifested in increased GGTP activity (>300% compared to the control, Fig. 2).

GGTP is a membrane-associated hepatocyte enzyme. The release of GGTP into the circulation serves as a marker of damage to the hepatocyte membrane. The 3-fold increase in plasma GGTP activity and activation of lipid peroxidation (LPO) suggest that cytolysis is associated with membrane LPO caused by activation of free radical processes, *i.e.* CAI is accompanied by the development of oxidative stress due to activation of free radical processes, which determine increased blood GGTP activity.

Catalase activity did not decrease and remained above the control level on day 7 after alcohol withdrawal. The signs of cytolysis were observed under these conditions. Plasma GGTP activity in rats exceeded the control level, but was lower than in animals receiving ethanol. The intensity of peroxi-

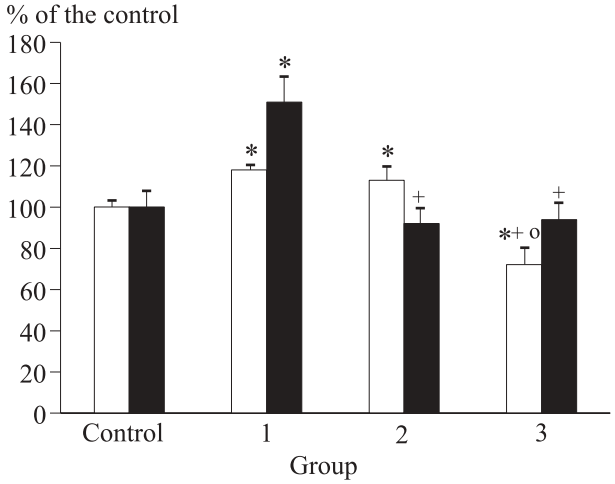

Fig. 1. Catalase activity (light bars) and concentration of TBA-reactive substances (dark bars) in the liver of rats after CAI, ethanol withdrawal, and PF administration during the withdrawal period. Here and in Figs. 2 and 3: *p<0.05 compared to the control; ^{+}p<0.05 compared to CAI; $^{\circ}p$<0.05 compared to the withdrawal period.

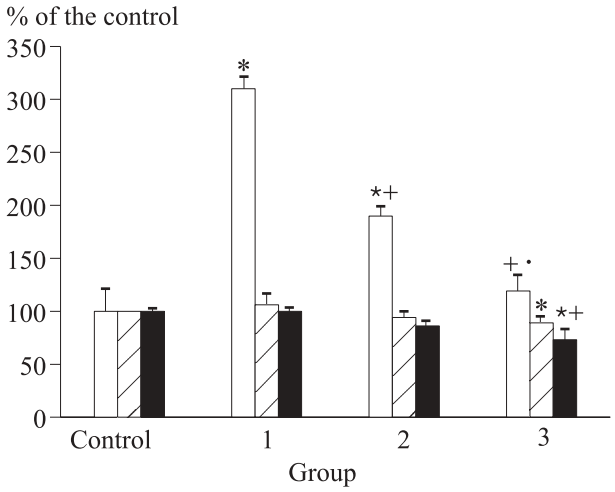

Fig. 2. Activities of GGTP (light bars), AST (shaded bars), and ALT (dark bars) in blood plasma from rats after CAI, ethanol withdrawal, and PF administration during the withdrawal period.

TABLE 1. Peroxidation Processes and Enzyme Activity in the Liver of Rats after CAI ($M\pm m$)

Parameter	Group			
	control	1	2	3
SOD, U/mg protein/min	596.20±123.50	593.70±166.70	600.50±142.30	583.00±139.60
Glutathione peroxidase, nmol glutathione/mg protein/min	1.44±0.16	1.65±0.15	1.52±0,13	1.18±0,19
Reduced glutathione, μmol/g	12.55±1.05	12.90±0.81	15.81±0.43*	12.81±0.76
Glutathione reductase, nmol $NADPH_2$/mg protein/min	0.29±0.03	0.37±0.05	0.24±0.02	0.26±0.04

Note. *p<0.05 compared to the control.

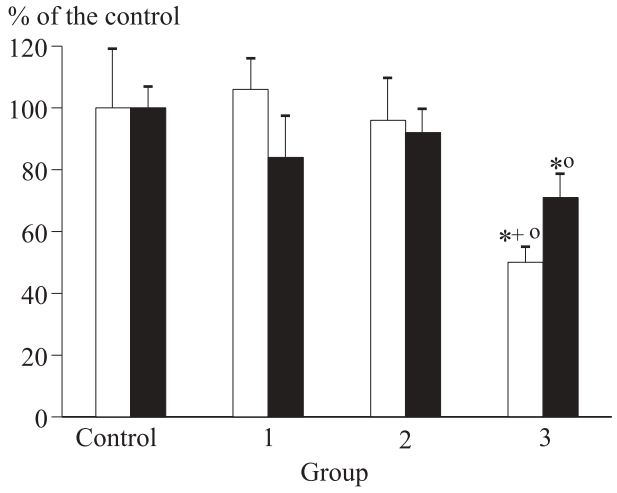

Fig. 3. NOx content in plasma (light bars) and liver (dark bars) from rats after CAI, ethanol withdrawal, and PF administration during the withdrawal period.

dation processes in these rats was lower than in group 1 animals and did not differ from the control.

PF prevented elevation of blood enzymes in CAI animals during the withdrawal period: GGTP activity was much lower than in animals of groups 1 and 2, plasma transaminase activity also decreased. The intensity of LPO decreased to the control level. We revealed a decrease in catalase activity. NOx concentration in blood plasma and liver decreased (Fig. 3).

The changes observed after PF administration were probably associated with the ability of perfluorocarbon micelles to adsorb O_2 and free radicals [11] initiating and maintaining free radical chain reactions. Catalase activity in PF-treated rats decreased compared to the control and other treatment groups, which was probably related to a decrease in the concentration of its substrate (H_2O_2). These data provide indirect support for our assumption. The decrease in the intracellular concentration of free radicals inhibits oxidation of polyunsaturated fatty acids in phospholipids of biological membranes. This conclusion was derived from the inhibition of peroxidation processes in PF-treated animals. The significant decrease in plasma transaminase activity is associated with the ability of perfluorocarbons to modify lipid composition and some physicochemical properties of the lipid bilayer [3,5]. PF probably modulated cell membrane permeability for enzymes (transaminase and GGTP) that can diffuse through these membranes under normal conditions.

Our results indicate that PF holds much promise for the prevention of CAI consequences.

REFERENCES

1. B. I. Islamov, Yu. V. Ladilov, V. A. Buevich, and R. V. Bobrovskii, *Vestn. Akad. Med. Nauk SSSR*, No. 3, 39-43 (1991).
2. A. Yu. Kovelenov, Yu. V. Lobzin, N. N. Pluzhnikova, and D. G. D'yachkov, *Eksper. Klin. Farmakol.*, **64**, No. 3, 41-44 (2001).
3. G. F. Leskova, A. B. Michunskaya, L. P. Kobozeva, and E. D. Klimenko, *Byull. Eksp. Biol. Med.*, **136**, No. 4, 340-343 (2003).
4. V. M. Moin, *Lab. Delo*, No. 12, 724-727 (1986).
5. E. R. Nagiev and M. M. Gazimagomedova, *Biomed. Khim.*, **49**, No. 2, 138-144 (2003).
6. S. Chevari, I. Chaba, and I. Sekei, *Lab. Delo*, No. 11, 678-681 (1985).
7. K. C. Lowe, *Oxygen Transport to Tissue*, Eds. M. Mochizuki *et al.*, New York (1988), Vol. X, pp. 655-663.
8. S. Marklund, I. Nordensson, and O. Back, *J. Gerontol.*, **36**, No. 4, 405-409 (1981).
9. H. Moshage, B. Kok, J. R. Huizenga, and P. L. Jansen, *Clin. Chem.*, **41**, No. 6, Pt. 1, 892-896 (1995).
10. M. Ohkawa, N. Oheshi, and K. Iaqi, *Anal. Biochem.*, **95**, No. 2, 351-358 (1979).
11. O. Rafikova, E. Sokolova, R. Rafikov, E. Nudler, *Circulation*, **110**, No. 23, 3573-3580 (2004).