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## PHARMACOLOGY AND TOXICOLOGY

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### Effect of Carnosine on Immunocompetent Cells from Alcoholic Patients

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We studied the effect of natural dipeptide carnosine on phagocytosis, respiratory burst in neutrophils, and subpopulation composition of lymphocytes from healthy donors and alcoholic patients. Carnosine *in vitro* produced different effects on immunocompetent cells from healthy donors and patients with alcoholism. In patients with alcoholism phagocytic activity of leukocytes and generation of reaction oxygen species increased under the influence of carnosine in a concentration of 0.01 mM, but decreased after treatment with this compound in a concentration of 1 mM. Carnosine in both concentrations stimulated the respiratory burst, but had no effect on the count of phagocytic cells in healthy donors. Carnosine in a concentration of 0.01 mM increased the number of lymphocytes carrying apoptosis markers (CD95<sup>+</sup>) in patients with alcoholism not receiving therapy. Our results indicate that carnosine holds much promise for the therapy of alcoholism.

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**Key Words:** *immunity; phagocytosis; neutrophils respiratory burst; carnosine; alcoholism*

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Experimental and clinical observations indicate that dipeptide carnosine produces a variety of effects. This compound has buffer and membrane-protecting properties. Antistress activity of carnosine is associated with its antioxidant effect. Carnosine is considered as a natural substance involved in hemostasis [2]. Chemiluminescence assay showed that carnosine and related dipeptides modulate activity of leukocytes in humans, rats, and rabbits [3,10,11,13]. The effect of carnosine on the respiratory burst in leukocytes depends on dipeptide concentration. Carnosine in a concentration of 1 mM inhibited generation of reactive oxygen species (ROS) by leukocytes. However, this process was activated under the influence of carnosine

in concentrations of 6-10 mM [10]. Previous studies showed that the inhibitory effect of carnosine on the respiratory burst is related to suppression of hypochlorite production. The activating effect of carnosine is realized via stimulation of superoxide anion generation [11,13].

*In vitro* studies of blood cells are usually performed with carnosine in high concentrations (1-10 mM) [8,11,13]. It should be emphasized that carnosine concentration in the blood from fasting healthy donors does not exceed 80 nM [2], which is associated with high activity of plasma carnosinase. Carnosine appears in the blood of people feeding meat-rich diet and patients with diseases accompanied by carnosinemia. The influence of carnosine in low concentrations ( $10^{-5}$  M) on blood immunocompetent cells is little studied. Moreover, the effects of carnosine on functional activity of leukocytes under normal and pathological conditions were never compared.

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Here we compared *in vitro* effects of carnosine in concentrations of 0.01 and 1 mM on blood immunocompetent cells from healthy donors and patients with alcoholism.

## MATERIALS AND METHODS

Experiments were performed with blood samples from 10 healthy donors and 22 patients with alcoholism. This disorder is accompanied by oxidative damage to cells and tissues and structural-and-functional changes in erythrocytes and leukocytes [4,8,14]. The age of patients and healthy donors (men) varied from 30 to 55 years. Patients with alcoholism were admitted to the Department of Addictive Disorders (Institute of Mental Health, Tomsk Research Center). The blood from patients was taken before (abstinence, admittance to the hospital) and after standard disintoxication therapy (remission).

Phagocytic activity of leukocytes was estimated by their ability to engulf melanin-formaldehyde latex particles. Phagocytic activity of cells was estimated by the phagocytic index (coefficient proportional to the percentage of phagocytic neutrophils) and phagocytic number (average number of latex particles engulfed by 1 phagocyte). The intensity of neutrophil respiratory burst was determined by the reduction of nitroblue tetrazolium to diformazan in the cell cytoplasm (NBT test). Melanin-formaldehyde latex served as a stimulator. We estimated the percentage of cells forming diformazan granules without stimulation (spontaneous NBT test for ROS generation in the absence of respiratory burst stimulators) and under the influence of melanin-formaldehyde latex (stimulated NBT test for the respiratory burst). The cytologic index (CI) was in

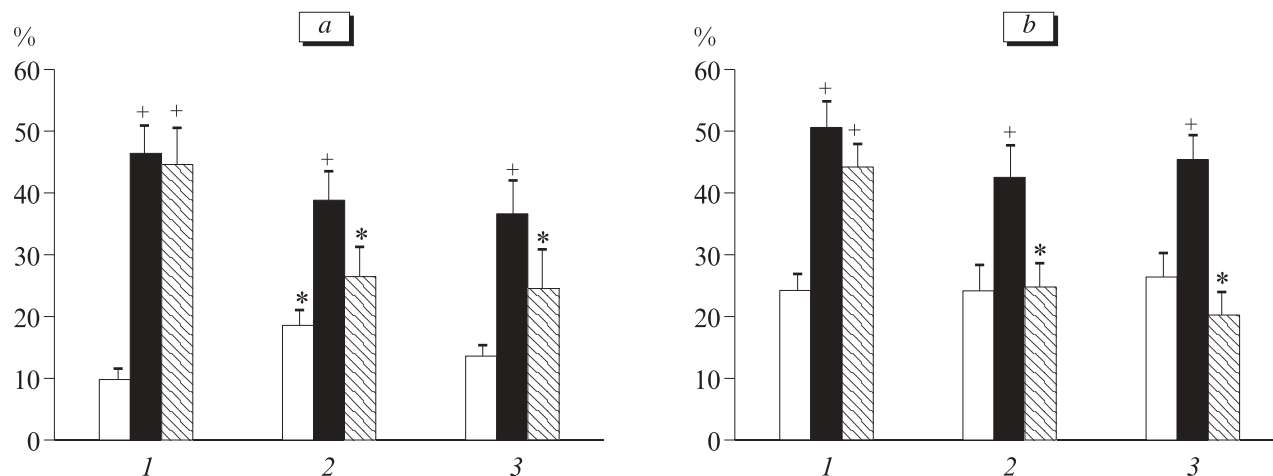
direct proportion to the number of diformazan granules formed in spontaneous and stimulated NBT test. Subpopulations of CD4<sup>+</sup> (T-helpers/inductors), CD8<sup>+</sup> (cytotoxic killers/suppressors), and CD95<sup>+</sup> lymphocytes (cells with apoptosis markers) were assayed in a fluorescence study with monoclonal antibodies [5].

Blood samples from untreated or treated patients and healthy donors (control, study of phagocytosis and neutrophil respiratory burst) or routinely isolated lymphocytes (study of subpopulations) were incubated in Hanks solutions without (baseline level) or with carnosine in concentrations of 0.01 or 1 mM at 37°C for 30 min. The results were analyzed by Student's *t* test and Mann—Whitney test.

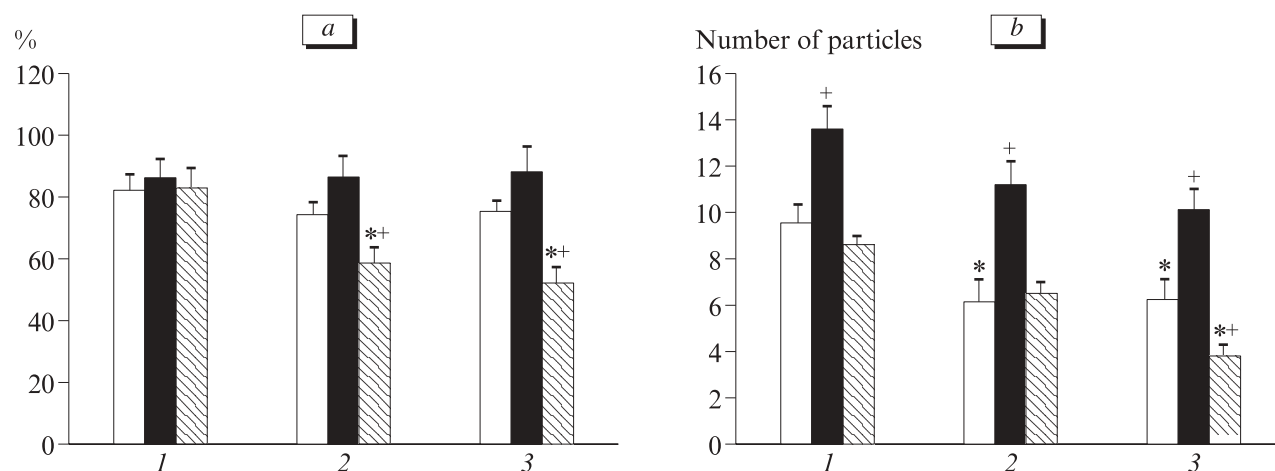
## RESULTS

Spontaneous NBT test showed that ROS generation by leukocytes significantly increased in alcoholic patients. After therapy the indexes of spontaneous NBT test approached the control level (Fig. 1, *a*). No differences were revealed in stimulated NBT test with cells from untreated or treated alcoholic patients and healthy donors (Fig. 1, *b*). The phagocytic number of neutrophils in patients with alcoholism was lower than in healthy donors (Fig. 2, *b*). We compared the results of spontaneous and stimulated NBT tests with cells from alcoholic patients and healthy donors. Leukocytes from abstinent alcohol drinkers were in the activated state and had reduced reserve capacity. Our results are consistent with published data that alcoholism is accompanied by oxidative stress [14] and functional changes in immunocompetent cells [4].

The effect of carnosine on phagocytic activity of neutrophils and ROS generation depended on its concen-



**Fig. 1.** Effect of carnosine on ROS generation by neutrophils (NBT test) from healthy donors (1) and patients with alcoholism in the stage of abstinence (2) and remission (3). Spontaneous NBT test, percentage of cells forming diformazan granules without stimulation (*a*); stimulated NBT test, percentage of cells forming diformazan granules after stimulation with latex (*b*). Here and in Fig. 2: light bars, baseline level; dark bars, 0.01 mM carnosine; shaded bars, 1.00 mM carnosine. Control (1) and before (2) and after therapy (3). \**p* < 0.05 compared to the control; +*p* < 0.05 compared to the baseline level.



**Fig. 2.** Effect of carnosine on phagocytic activity of neutrophils from healthy donors (1) and patients with alcoholism in the stage of abstinence (2) and remission (3). Phagocytic index (percentage of phagocytic neutrophils, a) and phagocytic number (average number of latex particles engulfed by 1 phagocyte, b).

tration. In a concentration of 0.01 mM carnosine increased phagocytic activity of polymorphonuclear leukocytes from healthy donors and patients with different stages of alcoholism (Fig. 2, b), while in a concentration of 1 mM it decreased the phagocytic index in alcoholic patients before and after therapy, but had no effect on these parameters in healthy donors (Fig. 2, a, b).

Carnosine in a concentration of 0.01 mM stimulated ROS generation in spontaneous and stimulated NBT test. This effect was observed in healthy donors and patients with different stages of alcoholism. Carnosine in a concentration of 1 mM activated ROS generation in healthy donors, but had no effect in patients with alcoholism (Fig. 1).

Depending on its concentration and functional state of neutrophils carnosine can act as an activator or inhibitor of the respiratory burst. Functional activity of neutrophils differs in patients with alcoholism and healthy donors, which is associated with chronic exposure to alcohol and its metabolites. *In vitro* studies showed that dipeptides thymogen, Vilon, and Bestim in low concentrations exhibit immunomodulatory activity [1,6,7,12].

Short peptides are considered as possible universal regulators of vital functions in the organism [1,9,12].

Subpopulations of lymphocytes were studied with carnosine in a concentration of 0.01 mM. No differences were found in the number of CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes in the presence of carnosine. However, incubation of lymphocytes from abstinent alcohol drinkers with carnosine was followed by an increase in the count of cells with apoptosis markers (CD95<sup>+</sup>, Table 1). The mechanism of this effect remains unclear. Previous studies revealed a relationship between suppression of ROS generation with 10 mM carnosine and apoptosis in rat neurons [2]. Our results indicate that carnosine in a concentration of 0.01 mM activates ROS generation by neutrophils and increases expression of apoptosis markers in pathologically modified immunocompetent cells from patients with alcoholism. Carnosine had no effect on cells from healthy donors and patients receiving therapy.

These data suggest that carnosine *in vitro* has a significant immunomodulatory effect on cells from abstinent alcohol drinkers. It manifested in the re-

**TABLE 1.** *In Vitro* Effect of 0.01 mM Carnosine on Subpopulations of Human Blood Lymphocytes (Number of Cells, %,  $M \pm m$ )

| Parameter         |                | Healthy donors (n=10) | Patients with alcoholism (n=22) |               |
|-------------------|----------------|-----------------------|---------------------------------|---------------|
|                   |                |                       | before therapy                  | after therapy |
| CD95 <sup>+</sup> | baseline level | 14.0±1.6              | 15.36±1.10                      | 21.0±1.9*     |
|                   | carnosine      | 18.22±1.90            | 21.5±1.8 <sup>+</sup>           | 21.75±2.00    |
| CD4 <sup>+</sup>  | baseline level | 31.2±2.2              | 33.45±3.80                      | 34.0±2.8      |
|                   | carnosine      | 33.34±2.30            | 41.0±4.2                        | 34.67±2.90    |
| CD8 <sup>+</sup>  | baseline level | 23.75±1.80            | 20.67±1.90                      | 26.5±1.7      |
|                   | carnosine      | 27.7±1.8              | 23.75±2.10                      | 27.75±1.80    |

**Note.** \* $p < 0.05$  compared to healthy donors; <sup>+</sup> $p < 0.05$  compared to the baseline level.

gulation of phagocytosis, ROS generation by neutrophils, and apoptosis. Therefore, carnosine holds much promise for the therapy of patients with alcoholism.

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