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Research Article

The Influence of Nitrile Acrylate Acute Intoxication on the Stem Cell Migration from Bone Marrow to Spleen and the Parameters of Innate and Adaptive Immunity

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Abstract

Experiments on random-bred albino mice and rats showed that nitrile acrylate acute intoxication (0.25 and 0.75 LD_{50}) dose-dependent reduces the integral state of the organism's anti-infection nonspecific resistance (innate immunity) due to suppression of bactericidal activity of blood serum, serum concentrations of lysozyme, platelet cationic protein, functional activity of neutrophils, increased the mortality rate of mice in comparison with the control and CFUs of opportunistic flora in peripheral blood and spleen. The action of nitrile acrylate in direct dose dependence reduces the stem cell migration from bone marrow to spleen and the basic parameters of innate and adaptive immunity.

Keywords: Nitrile Acrylate; Immunotoxicity; Stem Cell Migration; Innate Immunity; Adaptive Immunity; *E. Coli*

Introduction

Nitrile acrylate (NA, acrylonitrile, cyanoethylate, 2-propenitrile, cyan vinyl) is a volatile, colorless, flammable liquid mixed with most organic solvents. NA is widely used in industry as a raw material for the production of polyacrylonitrile and modacrylic yarns, synthetic rubbers, nitrile elastics, acrylamide and other materials. NA is referred to strong toxicants. Acute intoxication of NA is possible when the toxicant enters the body by inhalation, through the skin and the gastrointestinal tract [1-3]. In experiments on animals, it was demonstrated that NA intoxication is accompanied by lesions of almost all body systems and organs, initiation of lipid peroxidation, damage to DNA, increase in mutations in the chromosome genome by the implementation of carcinogenic action, and other toxic effects [1,2,4-8].

Acrylates, including NA, were included in the list of the priority chemical pollutants at the UNO Conference for Environment and Development (1992) [8]. Destruction of objects (reservoirs) containing NA (terrorist acts, accidents) can lead to serious ecological aftereffects. Territorial (soil, water) pollution is harmful for humans, animals, and plants because of suppression of humoral and cellular immune reactions (formation of secondary immunodeficiency) [1,2,8,9]. The study of violations of innate and adaptive immunity parameters, as well as the stem cell migration from bone marrow to spleen, is of both theoretical and practical interest for the prevention and treatment of various infectious complications and diseases arising after NA acute intoxication [1-3,9].

Aim of the Study

The aim of the study was to evaluate nitrile acrylate acute intoxication (0.25 and 0.75 LD_{50}) changes the stem cell migration from bone marrow to spleen and the basic parameters of innate and adaptive immunity.

Materials and Methods

The experiments were performed on random-bred albino rats and mice of both sexes weighing 180 - 240 g and 18 - 22 g respectively.NA (Sigma-Aldrich) was administered subcutaneously once a single dose of 0.25 and 0.75 LD_{50}. LD_{50} of NA for rats and mice after subcutaneous administration was 75 ± 8 and 36 ± 4 mg/kg, respectively. The study of the integral state of the organism's anti-infection nonspecific resistance (innate immunity) was determined by the indices of the experimental infection course caused by intra-

peritoneal injection of mice of the *E. coli* O157:H7 daily culture suspension in a single doses of 1.5×10^9, 2.0×10^9 and 2.5×10^9 CFUs in 1.3 - 2.0 ml of saline (sepsis modeling) [10,11,12]. Anti-infection nonspecific resistance (innate immunity) and the integral state of the innate immunity was evaluated by mortality of mice (*E. coli*, i.p.) during 36 h from experimental infection (sepsis modeling), as well as by mean lethal doses (LD_{50}) of *E. soli* and mean effective lifetime of animals (Et_{50}) in experimental and control groups calculated by probity analysis [10].

The effect of NA on stem cell migration from bone marrow to spleen was investigated by endogenous colony formation (number CFUs of spleen) after lethal irradiation of mice at a dose of 8 Gy when shielding of bone marrow mice to 1/2 of the shin by the number CFUs of spleen after 8 days [2,11,13]. NA was administered 30 minutes after the animals were irradiated.

The bactericidal activity of blood serum (BABS) of mice, serum concentrations of lysozyme, platelet cationic protein (PCP), functional activity of neutrophils in the test with nitro blue tetrazolium (NBT) and contents of *E. coli* of peripheral blood (CFUs in 0.05 ml) and spleen of surviving mice was determined after intraperitoneal administration of the *E. coli coli* O157:H7 daily culture suspension in a single doses of 2.5×10^9 CFUs in 2.0 ml of saline by conventional methods [2,11]. These parameters of innate immunity were evaluated 48 h after NA administration.

The parameters of adaptive immunity were evaluated by generally accepted methods in experimental immunotoxicology and immunology [2,11,14] The humoral immune response to T-dependent antigen (red sheep blood cells - RSBC), which characterizes the ability of Th1 cells to participate in the production of IgM by B cells (plasma cells), was determined by the number of antibody-forming cells (AFC) in the spleen 4 days after immunization (peak IgM production) which was administered intraperitoneally at dose of 2×10^8 RSBC. Similarly, we evaluated the humoral immune response to a T-independent typhoid Vi-antigen (Vi-Ag), reflecting the function of B cells and the synthesis of IgM by plasma cells (B cells) of the rat spleen. In this case, rats were immunized of Vi-Ag at a dose of 8 μg/kg [2,11,14].

The by delayed-type hypersensitivity (DTH) was studied in rats by weight gain of the hind paw foot in%. The resolving dose of RSBC (5×10^8) was administered under the aponeurosis of foot of the hind paw 4 days after immunization, which was performed intraperitoneally almost simultaneously with the administered of NA. The reaction of DTH was evaluated after 24 h [2,11].

The function of Th2 lymphocytes was investigated by the number of AFC, synthesizing IgG to RSBC, in the spleen after 13 days (peak IgG production [14]) after immunization 2×10^8 RSBC almost simultaneously with the administered of AN) by indirect local hemolysis in the gel [2,11,14]. Evaluation of the activity of natural killer cells (NK) and antibody-dependent cellular cytotoxicity (ADCC) was performed by the spectrophotometric method 4 days after the injection of NA [2,11].

The data obtained were processed statistically using the Student's t-test. Differences between the parameters were considered reliable at $p < 0.05$.

Results

Under the influence of NA acute intoxication at doses of 0.25 and 0.75 LD_{50} the mortality rate of mice increased in comparison with the control, respectively, in 1.40 (by 20%) ($p > 0.05$) and 1.60 times (by 30%) ($p < 0.05$), decreased LD50 *E. coli* in 1.65 ($p < 0.05$) and 1.97 times ($p < 0.05$) and Et_{50} mice, respectively, - in 1.34 ($p > 0.05$) and 1.52 times ($p < 0.05$), indicating innate immunity suppression. Under the influence of NA acute intoxication at doses of 0.25 and 0.75 LD_{50} the BABS of mice decreased, respectively, in 1.36 and 1.80 times ($p < 0.05$), serum concentrations of lysozyme - decreased in 1.29 ($p < 0.05$) and 1.69 times ($p < 0.05$), blood content of PCP decreased, respectively, in 1.25 and 1.38 times ($p < 0.05$). The functional activity of neutrophils (NBT test) decreased in 2.4 and 3.0 times ($p < 0.05$), respectively, while the content of *E. coli* of peripheral blood (CFUs in 0.05 ml) and the number of *E. coli* in spleen increased in 2.04 and 2.92 ($p < 0.05$), 1.64 and 2.28 times ($p < 0.05$), respectively (Table 1).

The stem cell migration from bone marrow to spleen of mice (number CFUs of spleen) after NA acute intoxication at doses of 0.25 and 0.75 LD50 decreased in 1.68 and 2.41 times ($p < 0.05$), respectively. The parameters of T-dependent - AFC to RSBC (IgM), AFC to RSBC (IgG) after NA acute intoxication at doses of 0.25 and 0.75 LD50 as well as the T-independent humoral immune response - AFC to Vi-Ag (IgM) decreased after 4 days, respectively, in 1.34 and 1.65 times ($p < 0.05$); in 1.50 and 1.73 times ($p < 0.05$); in 1.31 ($p > 0.05$) and 1.69 times ($p < 0.05$). The parameters of cellular immune reactions — the activity of NK, ADCC, DTH — after NA acute intoxication (0.25 and 0.75 LD50) decreased, respectively, in 2.13 and 2.78 times ($p < 0.05$); in 1.43 and 1.83 times ($p < 0.05$); 1.33 ($p > 0.05$) and 1.52 times ($p < 0.05$) (Table 2).

Parameters	Control	0,25 LD_{50}	0,5 LD_{50}
Mortality, %	50,0 ± 11,2 (20)	70,0 ± 10,2 (20)	80,0 ± 8,0* (25)
LD_{50} *E. coli*, 10^9 CFUs	1,73 ± 0,12 (20)	1,05 ± 0,10* (20)	0,88 ± 0,11* (20)
Et_{50}, h	17,2 ± 2,3 (20)	12,8 ± 2,2 (20)	11,3 ± 1,8* (25)
BABS, %	82,3 ± 3,6 (30)	60,5 ± 5,1* (15)	45,6 ± 6,6* (15)
Lysozyme, mg/l	7,1 ± 0,8 (30)	5,5 ± 0,8 (15)	4,2 ± 1,1* (15)
PCP, %	60,1 ± 2,3 (30)	48,1 ± 3,9* (15)	43,5 ± 3,2* (15)
Functional activity of neutrophils (NBT test)	0,24 ± 0,02 (30)	0,10 ± 0,02* (15)	0,08 ± 0,01* (15)
Content of *E. coli* of peripheral blood (CFUs in 0.05 ml)	25,0 ± 8,5 (7)	51,0 ± 10,1* (7)	73,0 ± 6,5* (6)
Number of *E. coli* in spleen, CFUs x 10^2	67,0 ± 10,1 (10)	110,0 ± 9,3* (10)	153,0 ± 10,9* (8)

Table 1: Effect of NA acute intoxication (0,25 и 0,75 LD50) on parameters of innate immunity of albino mice.

* -p <0,05 as compared to control; in parentheses - the number of animals.

Parameters	Control	0,25 LD50	0,75 LD_{50}
Number CFUs of spleen of mice	12,3 ± 3,2	7,3 ± 2,3	5,1 ± 2,0*
AFC to RSBC (IgM), 10^3	36,9 ± 4,0	27,5 ± 2,6*	22,3 ± 2,3*
AFC to RSBC (IgG), 10^3	50,1 ± 5,2	33,3 ± 3,8*	28,9 ± 3,0*
AFC to Vi-Ag (IgM), 10^3	23,7 ± 2,8	18,1 ± 1,9	14,0 ± 1,5*
NK activity, %	35,6 ± 2,6	16,7 ± 1,6*	12,8 ± 1,4*
ADCC, %	11,9 ± 1,4	8,3 ± 0,9*	6,5 ± 0,7*
DTH reaction, %	30,7 ± 3,1	23,1 ± 2,4	20,2 ± 2,1*

Table 2: The effect of NA acute intoxication (0,25 and 0,75 LD50) on the stem cell migration from bone marrow to spleen (number CFUs of spleen) of albino mice and the parameters of the adaptive immunity of albino rats (M ± m, n = 8-12).

* -p <0,05 as compared to control.

Discussion

Under the influence of NA acute intoxication the mortality rate of mice increased in comparison with the control, have been declining in comparison with the control the bactericidal activity of blood serum of mice, serum concentrations of lysozyme, blood content of platelet cationic protein, functional activity of neutrophils in NBT test and contents of *E. coli* of peripheral blood (CFUs in 0.05 ml) and spleen. The decrease in these parameters is directly related to the dose of NA. This indicates a decrease in the integral state of the organism's anti-infection nonspecific resistance (innate immunity) [1,2,3,9,11].

Dose-dependent decrease under the influence of NA the stem cell migration from bone marrow to spleen is caused by the defeat of these cells in the spleen by the toxicants and the disturbance of the stem cell migration nervous regulation [1,11].

After acute NA intoxication, the synthesis of antibodies to the thymus-dependent antigen (RSBC) and T-independent (Vi-Ag) antigens decreased. At the same time, the decrease in the Th2-dependent antibody product (AFC to RSBC (IgG) was more pronounced. The revealed decrease in the parameters of innate and adaptive immunity in case of acute NA intoxication may be associated with the lesion of blood cells producing nonspecific protective factors (BABS, PCB, etc.), changes in biochemical reactions and morphological structures of cells determining their resistance to infection [1,11].

Apparently, an important role in these processes is played not so much by NA, as by its toxic metabolites, in particular, hydrogen cyanide. Probably, it is hydrogen cyanide that determines the immunotoxin effect of NA by inhibiting the component of a3 cytochrome-oxidase in the system of tissue respiration enzymes of mitochondria of immunocompetent cells. It should be noted that hydrogen cyanide in the metabolism of NA (acute intoxication) enters the tissue respiration system of mitochondria of lymphoid cells within several hours (up to 24 hours). Violation of the T-dependent immune response to a greater extent than the T-independent one under the influence of NA is conditioned by its simultaneous action

on a greater number of cells participating in this immune reaction: macrophages, B lymphocytes and T cells. T-independent humoral immune response is known to be provided by the function of B cells activated by the antigen in the presence of IL-1, which is mainly secreted by macrophages [1,7,9,11].

It can be assumed that a T-dependent humoral immune response requires more energy, the main source of which is ATP, than a T-independent immune response. Due to inhibition of a3 component of tissue respiration system at acute NA poisoning, ATP production is likely to decrease significantly, which leads to a decrease in the synthesis of cyclic nucleotides (cGMP and cAMP), which are necessary for the implementation of proliferation and differentiation of immunocytes. The decrease in DTH reaction reflects the toxic effect of NA on cellular immunity and may be associated with a decrease in the activity of macrophages and T cells belonging to the subpopulation Th1, synthesizing IL-3, as well as IL-2, TNF-β (lymphotoxin α), γ-interferon [2,15].

The obtained results allow us to suppose that in the case of NA intoxication, one of the causes of teratogenesis may be infectious complications and diseases associated with a decrease in innate and adaptive immunity.

Conclusions

1. Nitrile acrylate acute intoxication (0.25 and 0.75 LD_{50}) dose-dependent reduces the integral state of the organism's anti-infection nonspecific resistance (innate immunity) due to suppression of bactericidal activity of blood serum, serum concentrations of lysozyme, platelet cationic protein, functional activity of neutrophils, increased the mortality rate of mice in comparison with the control and CFUs of opportunistic flora in peripheral blood and spleen.
2. The action of nitrile acrylate in direct dose dependence reduces the stem cell migration from bone marrow to spleen and the basic parameters of innate and adaptive immunity.

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