Changes in Nonspecific Resistance and Humoral and Cellular Immune Reactions after Acute Acetonitryl Poisoning

P. F. Zabrodskii and V. F. Kirichuk

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 5, pp. 548-550, May, 1998
Original article submitted April 23, 1997

Acute poisoning of rats with acetonitryl (0.3 and 0.8 LD_{50}) decreased the bactericidal activity of the blood serum, serum activities of lysozyme and β-lysines, functional activity of neutrophils, counts of splenocytes producing antibodies to T-dependent and thymus-independent antigens, natural and antibody-dependent cytotoxicities, antigen-presenting function of macrophages, and delayed type hypersensitivity. The content of corticosterone in the plasma decreased under the effect of acetonitryl after 2 days. The effects of acetonitryl are dose-dependent.

Key Words: *acetonitryl; nonspecific resistance; immunotoxicity; corticosterone*

Acetonitryl (AN, cyanmethane, methylcyanide) is widely used in chemical industry for the synthesis of a variety of compounds; it is a good solvent of organic substances, being less toxic than hydrocyanic acid. However, it is very hazardous because of probable environmental pollution which may result from accidents at chemical plants [5]. The data on the effects of acute poisoning with AN on nonspecific resistance and immunity are scarce [3]. Study of immunotropic effects of this compound may result in the development of methods for drug correction of disordered nonspecific and immunological resistance, adequate to immune homeostasis disorders induced by AN, which will be used for preventing and treating postintoxication complications and diseases.

We investigated changes in nonspecific resistance of the organism and humoral and immune reactions after acute poisoning with AN.

MATERIALS AND METHODS

Experiments were carried out on Wistar rats weighing 180-240 g. Acetonitryl was injected subcutaneously in doses of 0.3 and 0.8 LD_{50} (LD_{50} 1.9±0.2). The nonspecific resistance factors (serum bactericidal activity, serum lysozyme and β-lysine activities, neutrophil phagocytic activity in the nitroblue tetrazolium test assessed from the neutrophil activity index) were studied routinely 2 days after injection of AN. Humoral immune response to T-dependent (sheep red blood cells — SRBC) and T-independent (Vi-Ag) antigens was assessed after 5 days by the count of antibody-producing cells (APC) in the spleen [1,9] after injection of AN simultaneous with intravenous immunization of rats by these antigens in doses of 2×10^8 cells and 8 μg/kg, respectively. Natural cytotoxicity (activity of natural killer cells) was evaluated 48 h after injection of AN by the method described previously [2].

Antibody-dependent cell cytotoxicity was measured by spectrometry [4] 5 days after immunization of rats with 108 SRBC. Development of delayed type hypersensitivity was assessed from the increment in the weight of the hind paw. Animals were immunized by intravenous injection of 108 SRBC. The resolving dose of SRBC (5×10^8) was injected under hind paw aponeurosis after 4 days. Delayed type hypersensitivity was assessed after 24 h. Immunization was car-

Department of Toxicology, Saratov Medical University

0007-4888/98/0005-0487$20.00

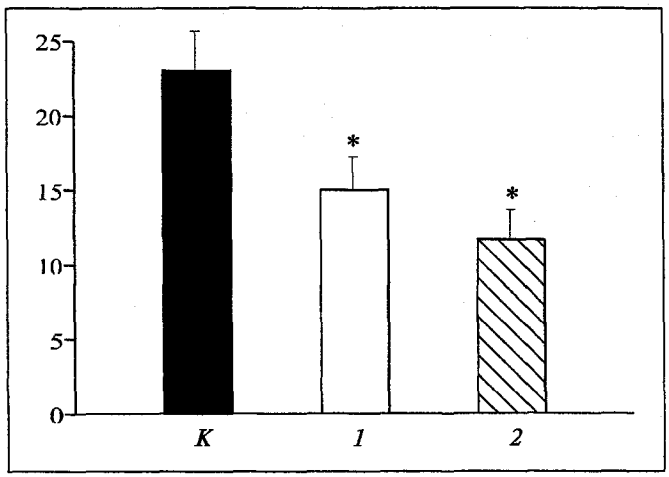

Fig. 1. Effect of acute acetonitryl poisoning on plasma content of corticosterone. Ordinate: corticosterone content, ng/ml. *K*) control; *1*) 0.3 L_{D50}; 2) 0.8 $L_{D5}0$. *$p<0.05$ *vs.* the control.

ried out simultaneously with AN injection. Macrophage ability to induce humoral immune response was studied as described previously [8]. Macrophages were isolated from the abdominal cavity of donor rats 2 days after injection of AN. Humoral immune response was assessed by the titer of antibodies to SRBC 5 days after injection of macrophages processing SRBC to recipient rats. Plasma corticosterone was measured by fluorometry [10] 2 days after AN intoxication. Data were statistically processed using Student's *t* test.

RESULTS

Two days after acute poisoning with AN, serum bactericidal activity, serum contents of lysozyme and β-lysines, and the neutrophil activity index decreased (Table 1). The reaction of these parameters directly depended on the dose of toxin. In a dose of 0.3 LD_{50} AN caused no statistically significant decrease in the content of β-lysines ($p<0.05$). It induced a decrease of humoral immune response to T-dependent (SRBC) and thymus-independent (Vi-Ag) antigens. Suppression of production of antibodies to these antigens was virtually the same (Table 2). However, in a dose of 0.8 LD_{50} the count of APC to SRBC in the spleen dropped 3.7 times, and to Vi-Ag 2.5 times. Acute AN poisoning led to an appreciable decrease in delayed type hypersensitivity, natural and antibody-dependent cytotoxicity, and decreased induction of humoral immune response by macrophages. Suppres-

Table 1. Changes in Nonspecific Resistance of Rats 2 Days after Acute Poisoning with AN (*M*±*m*)

Parameters	Control (*n*=30)	AN doses, LD_{50}	
		0.3 (*n*=15)	0.8 (*n*=15)
Serum bactericidal activity, %	82.3±3.6	64.5±5.9*	45.6±6.6*
Lysozyme, mg/liter	7.1±0.5	5.0±0.9*	4.2±1.1*
β-lysines, %	60.1±2.3	51.3±4.1	43.5±3.2*
Neutrophil activity index	0.24±0.02	0.12±0.02*	0.08±0.01*

Note. Here and in Table 2: *$p<0.05$ vs. the control.

Table 2. Effect of AN on Humoral and Cellular Immunity of Rats (*M*±*m*)

Parameters	Control (*n*=8-10)	AN doses, LD_{50} (*n*=7-8)	
		0.3	0.8
Titer of antibodies to SRBC, titer $-\log_2$	5.5±0.2	3.3±0.3*	2.8±0.2*
APC to SRBC, 10^3	25.5±4.1	12.4±2.3*	6.9±2.1*
APC to Vi-Ag, 10^3	23.1±3.2	13.4±2.9*	9.1±2.5*
Delayed type hypersensitivity reaction (increment of paw weight, %)	36.3±2.0	27.1±1.5*	21.3±1.8*
Cytotoxicity, %			
natural	29±6	17±4	11±5*
antibody-dependent	12.3±1.5	5.6±1.7*	3.8±1.1*
Induction of humoral immune response by macrophages, $-\log_2$ of antibody titer to SRBC	3.3±0.3	2.5±0.2*	2.0±0.3*

sion of cellular and humoral immunity under the effect of AN directly depended on its dose.

After 2 days, acute AN intoxication caused a dose-dependent decrease in plasma corticosterone level (Fig. 1).

These data imply that the decrease in nonspecific resistance and humoral and cellular immunity caused by AN is due to the general toxic effect of the toxin: inhibition of tissue respiration enzymes by cyanide in immunocyte mitochondria and disturbances in biochemical processes induced by AN and its toxic metabolites. The immunotoxic effect may result from direct action of biotransformation products or from secondary disorders caused by involvement of significant biochemical systems of spleen tissue and cells of other organs.

Our findings suggest that the immunodepressive effect of corticosterone is absent in AN intoxication.

Therefore, acute poisoning with AN inhibited humoral immune reactions (T-dependent humoral immune response is more suppressed), decreased the ability of macrophages to induce humoral immune response, and suppressed delayed type hypersensitivity and natural and antibody-dependent cytotoxicities. Impairment of nonspecific resistance and the decrease in cell-mediated and humoral immune responses are not caused by stress reaction (corticosteroids). These data prompt the use of the entire armory of immunostimulating drugs [6,7] for correcting immune homeostasis disorders after acute poisoning with AN.

REFERENCES

1. G. A. Belokrylov, V. Kh. Khavinson, and V. G. Morozov, *Zhu. Mikrobiol.*, No. 3, 97-99 (1980).
2. S. M. Gordienko, *Immunologiya*, No. 1, 31-36 (1984).
3. P. F. Zabrodskii, Med. *Truda Prom. Ekol.*, No. 5-6, 12-14 (1994).
4. Yu. I. Zimin and V. F. Lyakhov, *Immunologiya*, No. 1, 27-30 (1985).
5. R. Ludewig and K. Los, *Acute Poisonings* [in Russian], Moscow (1983).
6. K. D. Pletsityi and G. T. Sukhikh, *Dokl. Akad. Nauk SSSR*, **278**, No. 4, 1017-1019 (1984).
7. V. S. Shirinskii and E. A. Zhuk, *Immunologiya*, No. 3, 7-10 (1991).
8. B. F. Argyris, *Immunology*, **99**, No. 4, 744-750 (1967).
9. N. K. Jerne and A. A. Nordin, *Science*, **140**, No. 4, 405 (1963).
10. P. Moor, P. Osinski, R. Deckx, and O. Steeno, *Clin. Chim. Acta*, **7**, No. 4, 475-480 (1962).