Mechanisms of Immunotoxic Effects of Acrylonitrile

P. F. Zabrodskii, V. F. Kirichuk, V. G. Germanchuk, and V. G. Belikov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 129, No. 5, pp. 547-549, May, 2000
Original article submitted October 13, 1999

Cholinesterase reactivator dipyroxime and hydrogen cyanide antidote anticyan partially restore cell and humoral immune response in mice after acute acrylonitrile poisoning (0.5 LD_{50}). When used in combination these drugs virtually completely prevented the suppression of immune reactions. The main mechanism of immunotoxic effect of acrylonitrile is mediated through inhibition of T lymphocyte esterases and a_3 component of cytochrome *c* oxidase of immunocyte mitochondrial respiration enzymes, which is important for prevention and treatment of immune disturbances caused by this toxin.

Key Words: *acrylonitrile; immune response; delayed type hypersensitivity; α-naphthylbutyrate esterase*

Acrylonitrile (AN, acrylic acid nitril, vinylcyanide, propene nitril) is a highly toxic chemical substance used as raw material in the manufacture of polyacrylonitrile and modacryl fiber, synthetic rubbers, nitril elastic, acrylamide, glue, plexiglass, and other materials [7]. Chronic occupational exposure to AN apart from direct damage to organs and systems can cause dysfunction of the immune system [6]. In case of accident at a chemical plant, AN is extremely toxic as an environmental pollutant: it causes intoxications in the population, leading to development of syndromes characteristic of toxic compounds exerting general effects, and to postintoxication immunodeficiency [3].

We investigated the mechanisms of acute effect of AN on immune homeostasis.

MATERIALS AND METHODS

Experiments were performed on CBA mice (18-25 g). AN was injected subcutaneously in a dose of 0.5 LD_{50} (28±3 mg/kg for this mouse strain). One day after injection of AN, α-naphthylbutyrate esterase activity in mouse splenocytes was measured histochemically [5]. The results were correlated to delayed type hypersensitivity (DTH) reaction without immunocyte transfer and to adoptive DTH reaction involving cell transfer from donor mice to syngeneic recipients, and to the intensity of thymus-dependent humoral immune response evaluated by the number of antibody-producing cells in the spleen [8]. This humoral immune reaction characterizes the participation of type I T helpers (Th1) in IgM production by B lymphocytes (plasma cells). The formation of DTH reflecting the function of cell immune response (activity of Th1) was evaluated by mouse paw edema in comparison with the control. The animals were immunized intravenously with 2×10^8 sheep erythrocytes (SE) simultaneously with injection of AN. Secondary immune response was investigated by adoptive DTH reaction caused by splenocyte transfer (5×10^8) from syngeneic donor mice immunized with SE (10^8) to recipient mice. Recipient mice were sensitized after 1 h by intravenous injection of SE (10^7). The resolving dose of SE (5×10^8) was injected 4 days after immunization or sensitization under paw aponeurosis and the reaction was evaluated after 24 h. Splenocytes were collected 5 days after immunization of donors [4]. The role of α-naphthylbutyrate esterase in the formation of immunodeficiency was evaluated using cholinesterase reactivator dipyroxime. Antidotes of this group restore the activity of this enzyme after its inhibition with some toxins [1,2]. Dipyroxime was intraperitoneally injected to mice (including donor mice in DTH reaction) in a dose of 10 mg/kg immediately after AN injection, and 2 and

Department of Toxicology, Saratov Medical University

0007-4888/00/0005-463$25.00

24 h after it. The role of a_3 component of cytochrome *c* oxidase in the mitochondrial respiration enzyme system of immunocompetent cells was evaluated using hydrogen cyanide, a metabolite of AN, and its antidote anticyan. Anticyan acts via convertion of hemoglobin to methemoglobin. Methemoglobin reacts with cyan ion circulating in the blood and reactivates cytochrome a_3 by preventing binding of its trivalent iron with this ion. Anticyan was injected intraperitoneally (0.2 ml 1% solution) every 12 h for 2 days (maximal duration of AN biotransformation yielding hydrogen cyanide in mice [7]). The first injection was made 5 min after AN. Anticyan was used alone and in combination with dipyroxime. The results were statistically processed using Student's *t* test.

RESULTS

Acrylonitrile suppressed primary cell immune response, which was seen from a 2.54-fold inhibition of DTH reaction (Table 1). Humoral immune response, evaluated by the number of antibody-producing cells in the spleen, decreased 2.27 times. Dipyroxime partially restored these parameters, but they did not reach the control values ($p<0.05$).

Anticyan exerted a similar effect. Combined use of dipyroxime and anticyan completely restored cell and humoral immune reactions. Dipyroxime, but not anticyan restored the count of esterase-positive splenocytes, decreased by AN intoxication (Table 1). The effect of combined use of dipyroxime and anticyan was similar to that of dipyroxime alone (Table 1). Splenocyte transfer from immunized donors to recipients followed by injection of SE and evaluation of DTH reaction showed less pronounced paw edema under the effect of AN (Table 1). In this model the formation of DTH depended on immunogenic characteristics of donor splenocytes. Dipyroxime injected after AN partially restored the DTH reaction in this model. The effect of anticyan was similar. The combined use of the two agents virtually normalized secondary cell immune response, as evidenced by DTH test.

The immunotoxic effect of AN was abolishing due to reactivation of nonspecific esterases in donor mouse splenocytes with dipyroxime (Table 1). Naturally, the activity of transplanted splenocytes in DTH reaction increased. There are good grounds to consider that dipyroxime restored esterase activity in Th1 lymphocytes producing granulocytic macrophagal colony-stimulating factor, interleukins 2 and 3, interferon-γ, and necrosis factor-β (lymphotoxin) in the formation of DTH [9]. It is noteworthy that the increase in the count of antibody-producing cells in the spleen after dipyroxime is also associated with functional recovery of Th1 cells inducing IgM production by B lymphocytes.

Presumably, apart from α-naphthylbutyrate esterase, dipyroxime restores α-naphthyl-AS-acetate esterase inhibited by AN and acetyl cholinesterase, which are located mainly in T lymphocytes [5].

Hence, our findings suggest that immunotoxic effect of AN is realized via two main mechanisms: anticholinesterase activity and general toxicity, associated with inhibition of a_3 component of the immu-

TABLE 1. Effect of Intoxication with AN and Its Correction by Dipyroxime and Anticyan on DTH, Immune Response to SE, and Activity of Splenocyte α-Naphthylbutyrate Esterase (*M*±*m*, *n*=5-7)

Parameter	AN intoxication	Correction with		
		dipyroxime	anticyan	dipyroxime+ anticyan
Paw weight, mg				
without immunocyte transfer	38.9±3.8	38.9±3.8	32.7±3.2	34.1±3.1
	15.3±2.9*	38.9±3.8	23.0±2.8*+	32.4±2.8
splenocyte transfer after immunization	81.3±7.9	38.9±3.8	85.1±6.1	80.1±6.0
	55.3±4.7*	38.9±3.8	69.8±4.1*+	75.5±5.9
Number of antibody-producing cells in the spleen, $\times10^3$	34.3±3.5	34.3±3.5	34.3±3.5	34.3±3.5
	15.1±2.2*	24.8±2.8*+	21.1±3.0*	40.1±4.9
Number of esterase-positive splenocytes, %	39±3	39±3	36±4	42±4
	28±3*	35±3	25±3*	37±4

Note. $p<0.05$: *vs. the control, +vs. AN intoxication. Numerator: control; denominator: experiment.

nocyte respiration enzyme system by hydrogen cyanide. Anticholinesterase action of AN manifests in immunotoxic effect against T lymphocytes (esterases are located primariy in these cells), while inhibition of tissue respiration leading to immunosuppression is apparently typical of all immunocyte populations.

REFERENCES

1. P. F. Zabrodskii, *Byull. Eksp. Biol. Med.*, **121,** No. 4, 441-443 (1996).
2. P. F. Zabrodskii, *Eksp. Klin. Farmakol.*, **61,** No. 1, 57-59 (1998).
3. P. F. Zabrodskii and S. A. Romashchenko, *Ibid.*, No. 5, 56-58.
4. B. A. Frolov, V. N. Filipov, and R. Z. Meerson, *Immunologiya,* No. 2, 39-41 (1985).
5. F. G. Hayhow and D. Quaglino, *Hematological Histochemistry* [in Russian], Moscow (1983), pp. 156-176.
6. V. Ya. Shustov and I. S. Dovshanskii, *Gig. Truda,* No. 6, 18-21 (1987).
7. V. Ya. Shustov, A. G. Ol'khovskaya, and P. P. Kuznetsov, *Early Diagnosis and Prevention of Intoxications in the Manufacture of Viscose Cord, Capron, and Natron* [in Russian], Saratov (1985).
8. N. K. Jerne and A. A. Nordin, *Science,* **140,** No. 4, 405 (1963).
9. I. Kimber, *Experimental Immunotoxicology,* Eds. R. J. Smialowicz and M. P. Holsapple, New York, London (1996), pp. 391-417.