PHARMACOLOGY AND TOXICOLOGY

Changes in Nonspecific and Immune Resistance of the Organism in Acute Dichloroethane Intoxication

P. F. Zabrodskii, V. F. Kirichuk, and A. V. Gryzunov

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Experimental acute dichloroethane intoxication (0.75 LD_{50}) weakens the anti-infection resistance, suppresses antibody-dependent cell-mediated cytotoxicity, delayed-type hypersensitivity, and T-dependent humoral immune response and reduces the number of colony-forming units in the spleen. This suppression is probably associated with inhibition of α-naphthyl-AS-acetate esterase in immunocytes.

Key Words: *dichloroethane; nonspecific organism's resistance; immunotoxicity; α-naphthyl-AS-acetate esterase*

Considerable experimental and clinical evidence on the immunotropy of various toxins has been accumulated. However, the acute immunotoxicity of hepatotropic poisons (technical fluids) is poorly investigated. The only exception is the effect of carbon tetrachloride on humoral and cell-mediated immunity [2,5,9]. The effect of acute dichloroethane (DCE) intoxication on the nonspecific and immune resistance of the organism remains unclear [3]. Despite the low incidence of DCE intoxications (1-5%), investigation of the effect of DCE on the immune status is of great importance, because the mortality rate in DCE intoxication is rather high (32-96%) [6], presumably, due to post-intoxication immune deficiency leading to infections. Our goal was to examine the effect of acute DCE intoxication on nonspecific resistance and on the main factors of humoral and cell-mediated immunity.

Department of Toxicology, Saratov Medical University

MATERIALS AND METHODS

Experiments were carried out on CBA mice (18-22 g) and random-bred rats (180-240 g). DCE was administered *per os* in a dose of 0.75 LD_{50} (LD_{50}= 937±44 mg/kg). In rats, integral nonspecific and immune resistance were assessed by mortality (24 h after the treatment), mean lethal dose (LD_{50}) of *E. coli*, and mean effective lifetime (Et_{50}) in experimental peritonitis induced by a sublethal dose of *E. coli* (4, 6, or 7×10^9 microbial bodies) injected 3 days after immunization with the same culture (10^9 microbial bodies). The animals were immunized 30-60 min after administration of DCE. *E. coli* LD_{50} and Et_{50} were calculated using the probit-analysis. Nonspecific factors of the organism's resistance (serum antibacterial activity, serum lysozyme and β-lysine activities, neutrophil phagocytic activity in the nitro blue tetrasolium test, and colibacillus dissemination of peripheral blood (0.05 ml) and spleen in survived animals 48 h after peritonitis) were routinely determined 1, 3, 6, and 12 days postintoxication.

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TABLE 1. Effect of Acute DCE Intoxication on the Parameters of Resistance to Infection in Rats (7 Days After DCE Intoxication)

Parameters	Control	DCE
Mortality, %	12.5±5.1 (40)	32.4±7.5 (37)*
LD_{50} *E. coli*, 10^9 microbial bodies	9.0±1.0 (40)	7.5±1.2 (37)*
Et_{50}, hours	50.0±7.5 (40)	28.2±6.9 (37)*
E. coli dissemination of peripheral blood	33.0±9.8 (7)	61.0±5.9 (6)*
Number of *E. coli* in the spleen, 10^2	50.0±9.8 (10)	112.0±8.9 (8)*

Note. Here and in Table 3: the number of animals is shown in parentheses; *$p<0.05$ compared with the control.

The effect of DCE on endogenous colony formation was assessed in mice irradiated with a dose of 6.5 Gy as described elsewhere [15]. Humoral immune response to thymus-dependent (sheep erythrocytes) and T-independent (Vi-Ag) antigens was evaluated from the number of antibody-producing cells in the spleen [1,12] 5 days after DCE-treatment and simultaneous intravenous immunization with these antigens in doses of 2×10^8 cells and 8 μg/kg, respectively.

The antibody-dependent cell cytotoxicity (ADCC) was determined spectrophotometrically 5 days after immunization with 10^8 sheep erythrocytes [14]. The delayed-type hypersensitivity reaction (DTHR) in mice was assessed by the weight of the hinder paw. For this purpose the animals were immunized with sheep erythrocytes (10^8 cells intravenously). Four days later, the same antigen (5×10^8 cells) was injected under the aponeurosis plantaris of the hinder paw, and DTHR was evaluated after 24 h. Immunization was performed simultaneously with administration of DCE. The activity of α-naphthyl-AS-acetate esterase of mouse splenocytes was measured by the histochemical method [10]. The data were analyzed using Student's *t* test.

RESULTS

Acute DCE intoxication markedly increased the mortality rate in experimental rats and decreased LD_{50} of *E. coli* and Et_{50} (Table 1). The number of *E. coli* in the blood and spleen increased 1.8- and 2.2-fold, respectively, which was indicative of impaired nonspecific resistance. Thus, a reduction in immune resistance has been proved in rats, since the survival after a repeated injection of *E. coli* 3 days after immunization depends not only on the nonspecific defense factors but also on humoral and cell-mediated immunity. In acute DCE intoxication, serum antibacterial activity and phagocytic neutrophil activity remained significantly decreased during 3 days, while serum lysozyme and β-lysine contents were lowered for 6 days. On day 12, the nonspecific resistance parameters were practically the same as in the control (Table 2).

Impaired immune resistance of the organism in acute DCE intoxication is related to the inhibition of stem hemopoietic cell migration from the bone marrow to the spleen, the main reaction in the inductive phase of the immune response. In DCE-treated mice, the endogenous colony formation in the spleen was suppressed 1.8-fold (Table 3). DCE inhibited humoral immune response to thymus-independent and, to a higher extent, to T-dependent antigens. For instance, DCE inhibited the production of antibodies to sheep erythrocytes by 42% ($p<0.05$) and to Vi-Ag by 23% ($p>0.05$). Acute DCE intoxication also markedly reduced ADCC and DTHR.

The DCE-induced suppression of the thymus-dependent humoral and cell-mediated immunity may be mediated by inhibition of acethylcholinesterase and nonspecific esterases localized primarily in T

TABLE 2. Nonspecific Immunity of Rats after Acute DCE Intoxication

Parameter	Control (n=40)	Time after intoxication, days (n=10)			
		1	3	6	12
Serum antibacterial activity, %	85.0±4.3	48.3±7.5*	56.6±7.1*	68.4±7.8	78.8±7.3
Lysozyme, mg/liter	6.7±0.4	3.1±0.8*	3.3±0.9*	4.0±1.1*	6.0±1.2
β-Lysine, %	58.3±2.5	40.1±3.8*	35.2±4.2*	47.3±4.5*	54.2±4.8
Index of neutrophil activity (NBT-test)	0.21±0.02	0.06±0.01*	0.07±0.02*	0.18±0.02	0.23±0.03

Note. NBT: nitro blue tetrasolium. *$p<0.05$ compared with the control.

TABLE 3. Effect of Acute DCE Intoxication on the Main Immune Reactions in CBA Mice

Parameters	Control	DCE
Colony-forming units in the spleen	10.4±1.8 (15)	5.9±1.2 (10)*
Antibody-forming cells, 10^2:		
to sheep erythrocytes	27.8±3.2 (12)	16.1±2.8 (12)*
to VI-antigen	26.5±2.5 (12)	20.4±2.2 (12)
ADCC, %	8.8±1.5 (9)	5.0±0.9 (9)*
DTHR, %	37.5±2.1 (9)	31.3±1.5 (9)*
Esterase-positive splenocytes, %	48.0±3.0 (10)	35.0±2.0 (10)*

cells [13,14]. This hypothesis was confirmed by pronounced DCE-induced inhibition of α-naphthyl-AS-acetate esterase in splenocytes (Table 3). It should be noted that in control animals the number of esterase-positive cells in the spleen is consistent with the calculated number of T cells. Both T-cell depletion in lymphoid organs and inhibition of monocyte and macrophage esterases [10] may contribute to the postintoxication immune deficiency.

Thus, acute DCE intoxication impairs nonspecific resistance, particularly T-dependent humoral immune response, and suppresses the main reactions of immunocompetent cells. Our results suggest that T-dependent immune deficiency induced by acute DCE intoxication can be prevented by T-cell activators [8,11] and cholinesterase reactivators.

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