Anticholinesterase Mechanism as a Factor of Immunotoxicity of Various Chemical Compounds

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Experiments on Wistar rats showed that acute poisoning with chemicals in a dose of 0.75 LD_{50} (dimethyl dichlorovinyl phosphate, sarin, VX substance, sulfur yperite, lewisite, tetraethyl lead, dichloroethane) inhibiting platelet acetylcholine esterase, α-naphthyl-AS-acetate esterase, and α-naphthyl-butyrate esterase suppressed T cell-mediated immune reactions.

Key Words: *toxic chemicals; acetylcholine esterase; α-naphthyl-AS-acetate esterase; α-naphthyl-butyrate esterase; immunotoxicity*

Toxic chemicals with anticholinesterase effect are widely used in industry and agriculture. Apart from organophosphorus compounds and carbamic acid derivatives, acetylcholine esterase (ACE) and nonspecific esterases are inactivated by dichloroethane [4,7], tetraethyl lead [1,3], and other toxic agents, including military toxin agents now subject to neutralization, such as sarin (isopropylmethylfluorophosphonate), VX substance (phosphorylthiocholine derivative), sulfur yperite (2,2'-dichlorodiethylsulfide), and lewisite (β-chlorovinyldichlorarsine) [1,3]. Wide use of esterase inhibitors (including ACE inhibitors) in various spheres of human activities, utilization of organophosphorus compounds belonging to military toxins, and the risk of acute and chronic intoxications associated with this process prompt investigation of the role of ACE and nonspecific esterases in the realization of immunotropic effects of these chemicals. Postintoxication immunodeficiency can promote infectious complications and diseases [2,3]. In T lymphocytes ACE is located on the plasma membrane, while B cells are esterase-negative [3,13]. Studies of immunodeficiency caused by esterase inhibition (and hence, activation of the cholinergic and hypothalamic-pituitary-adrenal systems) are closely associated with investigation of the poststress changes of different etiology [6] and are of both theoretical and practical importance for the prevention and treatment of infectious complications and diseases.

We investigated the relationship between ACE-inhibitory effects of various chemical compounds and the realization of their T-cell immunotoxicity.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (180-220 g). Anticholinesterase effects (by ACE inhibition in T lymphocytes) of dimethyldichlorovinylphosphate (DDVP), sarin, VX substance, sulfur yperite, lewisite, dichloroethane, and tetraethyl lead were investigated after single subcutaneous injection of these agents in a dose of 0.75 LD_{50}. ACE activity in T lymphocytes was measured 4 days after intoxication. The cells were isolated by filtering splenic suspension through Nylon cotton (Nitron) [9]. The tests and estimations were carried out as described previously [13]. The amount of acetylcholine (in μmol) hydrolyzed per 1 min in 1 ml of suspension containing 10^9 T lymphocytes was taken for 1 unit of ACE activity. Activities of α-naphthyl-AS-acetate esterase and α-naphthyl-butyrate esterase in splenocyte suspension (mainly T cell) were measured by a histochemical method [8] 4 days after intoxication. T-dependent hu-

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moral immune reaction and function of Th1 lymphocytes were studied using delayed-type hypersensitivity (DTH) test. Humoral immune response was evaluated 4 days after intoxication by the number of antibody-producing cells (APC) in the spleen [10] after intraperitoneal immunization with thymus-dependent antigen (sheep erythrocytes, SE, 2×10^8 cells) 30 min after injection of the toxin. This test reflects production of IgM by splenic B cells with participation of Th1 lymphocytes. DTH characterizing the function of Th1 cells (and macrophages) in cellular immune reaction [5] was evaluated by the increase of the hind paw weight after intraperitoneal immunization with 10^8 SE 30 min after injection of the toxin. The challenge dose of SE (5×10^8 cells) was injected under hind paw aponeurosis after 4 days. DTH reaction was evaluated after 24 h.

The data were processed statistically using Student's *t* test.

RESULTS

T lymphocytes isolated from the spleen after acute intoxication with various toxic agents (0.75 LD_{50}) were characterized by lowered anticholinesterase activity compared to the control (Table 1). Acute poisoning with DDVP, sarin, VX substance, sulfur yperite, lewisite, tetraethyl lead, and dichloroethane significantly decreased ACE activity in splenic T lymphocytes (by 4.95, 6.87, 8.00, 4.45, 1.56, 1.45, and 1.39 times, respectively).

Inhibition of ACE in T cells and the decrease in the number of esterase-positive cells characterizing activity of nonspecific esterases in splenic T lymphocytes (and, to a certain extent, in monocytes and macrophages [8]) directly correlated with suppression of T-dependent antibody production and to the degree of DTH reduction. The number of APC to SE after intoxication with DDVP, sarin, VX substance, sulfur yperite, lewisite, tetraethyl lead, and dichloroethane decreased significantly 4 days postinjection (by 1.80, 2.30, 2.63, 4.08, 3.33, 1.69, and 1.54 times, respectively), DTH decreased by 1.39, 1.74, 1.82, 2.46, 2.11, 1.70, and 1.47 times, respectively ($p<0.05$).

DDVP, sarin, VX substance, sulfur yperite, lewisite, tetraethyl lead, and dichloroethane decreased the relative count of esterase-positive cells (T cells) in the spleen (Table 2) [5,11]. The relative count of T cells containing α-naphthyl-AS-acetate esterase significantly decreased 4 days after acute intoxication with DDVP, sarin, VX substance, sulfur yperite, lewisite, tetraethyl lead, and dichloroethane (by 1.89, 2.82, 3.29, 1.47, 1.34 ($p<0.05$), 1.24, and 1.20 times, respectively, $p>0.05$). The counts of α-naphthylbutyrate esterase-positive T lymphocytes decreased by 1.86, 2.72, 3.15, 1.45 ($p<0.05$), 1.30, and 1.21 times ($p>0.05$), respectively.

No doubt, ACE inhibition by toxic agents in sublethal doses plays a role in the formation of postintoxication immunodeficiency. This presumably involves the loss of some functions by T lymphocytes (*e.g.* by Th1 cells), which leads to attenuation of T-dependent immune reactions. This can be explained by excessive acetylcholine stimulation of muscarinic and nicotinic receptors, whose presence on T lymphocytes is proven [12], as a result of which the optimal cAMP to cGMP ratio in immunocytes, essential for their proliferation and differentiation, is distorted [12]. The role of nonspecific esterases in the realization of T-dependent immune reactions remains unclear, despite well-known possibility of their inhibition by anticholinesterase compounds. Of course, anticholinesterase effect is not the main mechanism in the realization of immunotoxic effects of yperite, lewisite, TEL, and DCE, in comparison with other numerous mechanisms of their effect, but this mechanism remains significant in the formation of T-cell immunodeficiency [1,3,4,7].

Published data indicate that organophosphorus compounds (and, as our studies showed, yperite, lewi-

TABLE 1. Effects of Toxic Agents (0.75 LD_{50}) on ACE Activity in Splenic T Cells and T-Dependent Immunity Reactions in Wistar Rats 4 Days after Acute Intoxication ($M\pm m$, n=9-11)

Chemical	Spleen, ACE activity, mUnits/10^9 cells	APC and SE, 10^3 cells	DTH reaction, increase in hind paw weight, %
Control	58.4±6.5	34.7±3.3	27.8±2.1
DDVP	11.8±2.7*	19.3±2.2*	20.0±2.5*
Sarin	8.5±2.1*	15.1±2.1*	16.0±1.7*
VX substance	7.3±1.7*	13.2±1.7*	15.3±1.8*
Yperite	32.5±4.3*	8.5±1.5*	11.3±1.6*
Lewisite	37.5±4.5*	10.4±2.0*	13.2±1.4*
Tetraethyl lead	40.1±3.8*	20.5±1.9*	16.4±1.5*
Dichloroethane	42.0±4.1*	22.6±2.4*	18.9±1.9*

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

TABLE 2. Effect of Acute Poisoning with Toxic Chemicals (0.75 LD_{50}) on the Counts of α-Naphthyl-AS-Acetate Esterase-Positive and α-Naphthylbutyrate Esterase-Positive Cells in Splenocytes of Wistar Rats (*M*±*m*, *n*=9-11)

Toxic Chemical	Content of α-naphthyl-AS-acetate esterase-positive cells, %	Content of α-naphthylbutyrate esterase-positive cells, %
Control	49.4±3.5	38.1±3.2
DDVP	26.1±2.7*	20.5±2.3*
Sarin	17.5±2.3*	14.0±2.0*
VX substance	15.0±1.9*	12.1±1.8*
Yperite	33.6±3.4*	22.9±2.7*
Lewisite	36.8±3.3*	26.2±2.5*
Tetraethyl lead	39.9±3.1	29.4±3.0
Dichloroethane	41.2±3.6	31.4±2.8

site, tetraethyl lead, and dichloroethane) inhibiting esterase activity in T lymphocytes, monocytes, intact and activated by lymphokines NC impair immunological regulation and effector functions mediated by these cells [11]. It was hypothesized that esterase inactivation in immunocompetent cells reduces esterase-dependent detoxification and initiates lymphomogenesis. In addition, the decrease in esterase activity suppresses immunity to Epstein—Barr virus and human herpes virus 6, promoting the development of lymphomas [11].

Hence, intoxication with toxic chemicals possessing anticholinesterase effects in sublethal doses leads to inhibition of ACE and nonspecific esterases in T lymphocytes, which results in suppression of T-dependent immune reactions.

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