PHARMACOLOGY AND TOXICOLOGY

Role of Cholinergic and Cytokine Regulation of T Cell Function in Stimulation and Inhibition of Immune Reactions in Intoxication by Organophosphorus Compounds in Different Doses

P. F. Zabrodskii, V. F. Kirichuk, V. G. Lim, I. H. Yafarova, and A. V. Kuzmin

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Experiments on outbred albino rats showed that administration of acetylcholine and aceclidine in a dose of 0.1 LD_{50} for 3 days and of dimethyl dichlorovinyl phosphate (organophosphorus compound) in a single dose of 0.05 LD_{50} stimulated the function of Th1 and Th2 lymphocytes and cytokine production by these cells. Dimethyl dichlorovinyl phosphate in a single dose of 0.5 LD_{50} produced an opposite effect. Acetylcholine and aceclidine stimulated activity of acetylcholinesterase in T cells, while dimethyl dichlorovinyl phosphate in a single dose of 0.5 LD_{50} inhibited it. During acute intoxication, the organophosphorus compound, depending on the dose, can stimulate (acetylcholine effect) and inhibit the immune reactions (acetylcholinesterase inhibition of T cells).

Key Words: *cholinomimetics; organophosphorus compound; immunotoxicity; Th1, Th2 lymphocytes; cytokines*

Numerous anticholinesterase substances are used in agriculture, in various branches of industry, and in medicine. These are mainly organophosphorus compounds. Wide use of these toxicants can lead to environmental pollution and cause intoxication in humans and animals [3,4]. Accidents can emerge at plants engaged in detoxication of chemical weapons, for example, organophosphorus compounds from the category of military toxins [2,4]. These accidents can result in release of organophosphorus compounds into the environment and intoxication of the stuff members of these plants, as well as of the population of the adjacent territories [5]. The need in studies of the mechanisms of formation of the postintoxication immunodeficiency after poisoning with organophosphorus compounds causes no doubt, as it isessential for prevention and treatment of the resultant infectious complications and diseases [4].

The development of highly effective antidotes for military organophosphorus intoxication control is in progress in foreign countries [9]; their delayed effects are analyzed [13]. This is explained by the possibility of using chemical weapons in terrorist and criminal acts [4,5] and in local military conflicts [12].

The study of immunotropic characteristics of organophosphorus compounds necessitates the study of the role of cholinergic receptors in the immunocom-

Saratov State Medical University, Russia. ***Address for correspondence:*** pz@renet.com.ru. P. F. Zabrodskii

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petent cell functioning and of their relationship with the cytokine production by T-cells [1]. Activation of muscarinic cholinergic receptors on T cell is associated with an increase in the production of IFN-γ and IL-4 [4], stimulating, respectively, the cellular (and IgM synthesis) and humoral immune reactions (production of IgG and other classes of immunoglobulins) [10,11], while inhibition of T-cell acetylcholinesterase is associated with inhibition of their function and the immune response linked with them [3]. Presumably, because of the probability of realization of these opposite (by their consequences) effects of organophosphorus compounds, in some cases these compounds stimulate (but not inhibit) the humoral and cellular immune reactions [4].

We evaluated the possibility of stimulation and suppression of humoral and cellular immune response under the effect of organophosphorus compounds, depending on the proportion of activation of T-cell muscarinic reactive structures and relevant production of cytokines, on the one hand, and degree of inactivation of acetylcholinesterase in Th1 and Th2 lymphocytes, on the other.

MATERIALS AND METHODS

Experiments were carried out on male and female outbred albino rats (180-240 g). One day after intraperitoneal immunization of animals with sheep erythrocytes (SE; 2×10^8 cells), the rats were injected subcutaneously with aceclidine (AC; muscarinic receptor agonist) in a single dose of 0.1 LD_{50} daily for 3 days. Acetylcholine chloride (AcC) was injected subcutaneously in a dose of 0.1 LD_{50} twice a day (because of rapid hydrolysis) for 3 days, also 1 day after immunization with SE. LD_{50} for AC and AcC (subcutaneous injections) were 4.1±0.2 and 215±18 mg/kg, respectively. The organophosphorus compound dimethyl dichlorovinyl phosphate (DDVP) was injected subcutaneously in single doses of 0.05 and 0.5 LD_{50} (LD_{50} 64.5±2.3 mg/kg) 2 days after rat immunization, that is, during the productive phase of immunogenesis. Controls were immunized with SE and after 1 day were subcutaneously injected with 0.5 ml isotonic NaCl in a volume equivalent to the volume of cholinergic substances solutions in experimental groups. The immunity parameters were evaluated by common methods of experimental immunotoxicology [2-4,6]. The function of Th1 lymphocytes was evaluated by the humoral immune reaction to T-dependent antigen (number of antibody-producing cells (APC), synthesizing IgM to SE, in the spleen) on day 5 after immunization with SE [4,10,11] and by delayed-type hypersensitivity (DTH) reaction. The formation of DTH reaction in animals was evaluated by the increment in the weight of the hind paw (in %). The resolving dose of SE (5×10^8 cells in 0.5 ml isotonic NaCl) was injected to control and experimental rats under the hind paw aponeurosis on day 4. DTH reaction was evaluated after 24 h. The function of Th2 lymphocytes was evaluated on day 8 by the number of APC to SE in the spleen by indirect local hemolysis in gel, characterizing the production of IgG [6,14].

The concentrations of IFN-γ and IL-4, produced by Th1 and Th2 lymphocytes , respectively [6,7], were measured in rat plasma by ELISA [6] using ELISA kits (BioSource Int.) on days 5 and 8 after the first injection of cholinergic substances (1 day before injection of cholinergic substances control and experimental rats were immunized with SE). Since the changes in cytokine concentrations on days 5 and 8 after the first injection of cholinergic substances and in the control were negligible, their mean plasma levels in the control were estimated.

Inhibition of T cell acetylcholinesterase with DDVP in doses of 0.05 and 0.5 LD_{50} was studied. Acetylcholinesterase activity in T cells was evaluated on day 5 after injection 1 of cholinergic substances. The cells were isolated from spleen suspension by filtration through Nylon cotton (Nitron) [8]; the reactions and estimations were carried out as described previously [15]. A micromole of acetylcholine, hydrolyzed within 1 min in a ml of suspension containing 10^9 T cells, was taken for a unit of acetylcholinesterase activity.

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

RESULTS

Injection of AcC (Table 1) stimulated the humoral immune response to T-dependent antigen (APC count in the spleen) characterizing the production of IgM and the function of Th1 lymphocytes and the formation of DTH on day 5 in comparison with the control 1.37 and 1.30 times, respectively ($p<0.05$). On day 8 after immunization, the production of IgG increased (evaluated by APC count in the spleen) 3.37 times ($p<0.05$) indicating an increase in Th2 lymphocyte activity. Similar results were observed after AC injection.

These data indicate that cholinoceptor agonists in a dose of 0.1 LD_{50}, approximately corresponding to the therapeutic single dose for humans, stimulate the function of Th1 and Th2 lymphocytes in rats.

Injection of DDVP in the minimum dose (0.05 LD_{50}) stimulated significantly the functions of Th1 and Th2 lymphocytes. The T-dependent antibody production, DTH reaction, and the counts of APC to SE, indicating the intensity of IgG production, increased 1.30, 1.27, and 1.32 times, respectively ($p<0.05$).

TABLE 1. Effects of Cholinergic Substances on the Function of Th1 and Th2 Lymphocytes in Rats (*M*±*m*; *n*=9-11)

Group	Th1 lymphocyte function		Th2 lymphocyte function
	APC to SE (IgM), 10^3	DTH, %	APC to SE (IgG), 10^3
Control	48.2±4.0	38.0±3.1	17.1±1.6
AcC, 0.1 LD_{50}	65.9±5.4*	49.4±4.3*	23.4±2.3*
AC, 0.1 LD_{50}	68.5±6.1*	56.6±5.2*	26.8±2.1*
DDVP, 0.05 LD_{50}	62.8±5.5*	48.3±4.2*	22.5±2.0*
DDVP, 0.5 LD_{50}	11.4±1.3*	19.8±2.0*	12.2±1.5*

Note. Here and in Tables 2, 3: *p<0.05 compared to the control.

In the maximum dose used in the study (0.5 LD_{50}) DDVP inhibited activities of the studied T-cell subpopulations (reducing the splenic count of APC to SE 4.23 times (p<0.05), suppressing the DTH reaction and IgG production (evaluated by the count of APC to SE) 1.92 and 1.40 times, respectively (p<0.05).

Hence, irreversible inhibitors of cholinesterase, for example, DDVP used in a dose of 0.05 LD_{50}, stimulate activities of Th1 and Th2 lymphocytes in rats due to the AcC effect. In doses higher than 0.05 LD_{50} and close to 0.5 LD_{50} they inhibit the functions of these T-cell subpopulations by inhibiting acetylcholinesterase on their cell membranes [4,15].

Measurements of plasma concentrations of cytokines (Table 2) on days 5 and 8 after immunization and subsequent AcC treatment showed elevated concentrations of IFN-γ (by 1.53 and 1.51 times, respectively; p<0.05) and of IL-4 (by 1.25 and 1.29 times, respectively; p<0.05). Similar changes were observed after injection of muscarinic receptor agonist AC. Injection of DDVP in a dose of 0.05 LD_{50} stimulated the production of IFN-γ by 1.28 and 1.29 times on days 5 and 8 after immunization (p<0.05) and of IL-4 by 1.10 and 1.16 times, respectively (p>0.05). These data confirm stimulation of Th1 and Th2 lymphocyte activities by cholinoceptor agonists in a dose of 0.1 LD_{50} and by DDVP in a dose of 0.05 LD_{50}.

The increase of the IFN-γ/IL-4 proportion characterizes the increase of Th1 lymphocyte functional activity in comparison with Th2 cells, while the decrease of this proportion indicates greater suppression of Th2 lymphocyte activity *vs.* Th1 cells [6,7]. The IFN-γ/IL-4 proportion after injections of AcC, AC, and DDVP in the minimum dose was 8.3, 8.5, and 7.9 after 5 days and 8.0, 8.7, and 7.7 after 8 days, respectively (6.8 in the control). The maximum DDVP dose led to a reduction of the IFN-γ/IL-4 proportion after 5 and 8 days 5.5 and 5.1 times, respectively. This indicates that cholinoceptor agonists and DDVP in doses of 0.1 and 0.05 LD_{50} stimulate activity of Th1 cells more intensely than of Th2 lymphocytes, while DDVP in a dose of 0.5 LD_{50} inhibits the function of Th1 cells. The

TABLE 2. Effects of Cholinergic Substances on Rat Plasma Cytokine Concentrations (*M*±*m*; *n*=7; pg/ml)

Substance	Day after immunization	IFN-γ	IL-4	IFN-γ/IL-4
Control (*n*=14)	—	856±71	126±12	6.8
AcC, 0.1 LD_{50}	5	1307±75*	158±17*	8.3
	8	1294±85*	162±15*	8.0
AC, 0.1 LD_{50}	5	1406±87*	166±16*	8.5
	8	1320±90*	171±15*	7.7
DDVP, 0.05 LD_{50}	5	1097±83*	139±14	7.9
	8	1107±89*	146±13	7.6
DDVP, 0.5 LD_{50}	5	438±42*	80±7*	5.5
	8	429±37*	84±8*	5.1

TABLE 3. Effects of Cholinergic Substances on Acetylcholinesterase Activity in Rat Splenic T Cells on Day 5 after Immunization (*M*±*m*; *n*=9-13)

Substance	Acetylcholinesterase activity, U/10^9 T-cells
Control	52.2±4.5
AcC, 0.1 LD_{50}	70.4±6.4*
AC, 0.1 LD_{50}	67.8±5.8*
DDVP, 0.05 LD_{50}	47.6±4.9
DDVP, 0.5 LD_{50}	18.7±2.0*

increase in Th1 cell activity in comparison with Th2 lymphocytes under the effect of AcC and AC suggests the presence of numerous muscarinic receptors on Th1 lymphocyte membrane.

After treatment with AcC and AC, T cells isolated from the spleen caused a statistically significant increase in acetylcholinesterase activity in T cells (1.35 and 1.30 times, respectively; $p<0.05$; Table 3). Treatment with DDVP in the minimum dose virtually did not reduce the content of acetylcholinesterase in T cells, while the dose of 0.5 LD_{50} caused a significant reduction (2.79 times; $p<0.05$) of acetylcholinesterase activity in T cells.

These results indicate that after treatment with AcC and AC acetylcholinesterase activity in T-cells directly correlated with the immune values, while in DDVP intoxication (0.5 LD_{50}) this correlation was inverse.

Injections of AcC and AC stimulated acetylcholinesterase activity in T-cells presumably by realization of the mechanisms maintaining the immune homeostasis through stimulation of AcC hydrolysis on T cell membrane. This led to a reduction of the effect of this mediator on muscarinic and nicotinic cholinergic receptors, whose presence on T cells is a proven fact [1].

The suppressive effect of organophosphorus compound in high doses on mainly Th1 lymphocytes can be explained by a significant increase in blood concentration of corticosterone [4], to which Th1 lymphocytes are more sensitive than Th2 cells [6].

Hence, AcC, AC, and DDVP in a dose of 0.05 LD_{50} stimulate the function of Th1 and Th2 lymphocytes and cytokine production by them. The effect of DDVP in dose of 0.5 LD_{50} was opposite. Aceclidin and AcC stimulated activity of acetylcholinesterase in T cells, DDVP in a dose of 0.05 LD_{50} did not modify it, while in a dose of 0.5 LD_{50} reduced it. Two major opposite effects can be realized in DDVP intoxication, depending on its dose: stimulation of immune reactions resultant from AcC effect on the T-cell muscarinic cholinergic receptors and inhibition of the immune response as a result of T cell membrane acetylcholinesterase inhibition (as a rule, the stimulatory effect of an organophosphorus compound on immunocompetent cells is realized at the toxicant doses several-fold lower than the mean lethal doses).

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