Role of Anticholinesterase Mechanism in Suppression of Antibody Formation during Acute Poisoning with Organophosphorus Compounds

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

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Experiments on Wistar rats showed that acute poisoning with organophosphorus compound dimethyl dichlorovinyl phosphate (0.5 LD_{50}) was accompanied by two opposite effects: inhibition of acetylcholinesterase in T lymphocytes leading to suppression of thymus-dependent antibody formation (predominant effect) and acetylcholine-induced stimulation of antibody production. Acetylcholine activated acetylcholinesterase in intact T cells.

Key Words: *organophosphorus compounds; acetylcholine; acetylcholinesterase; T lymphocyte; antibody formation*

Wide use of organophosphorus compounds (OPC) in agriculture and chemical industry, destruction of OPS belonging to chemical warfare agents, and the risk of acute or chronic OPC intoxication dictate the necessity of studying specific and nonspecific mechanisms underlying postintoxication immune deficiency for preventing and treating various infectious complications [3]. The immunotropic effects of OPC are mediated by the anticholinesterase mechanism, which includes inhibition of esterases in immunocompetent cells [2,3,7], inactivation of acetylcholinesterase (AChE), and accumulation of acetylcholine (ACh) in cholinergic synapses [3,13]. However, the role of AChE localized on the surface of T lymphocytes [10,14] and the contribution of high ACh concentration in the blood and lymphoid organs into antibody formation are poorly understood [2,3]. Unlike T lymphocytes carrying AChE on the cell membrane, B cells are esterase-negative [4,10,14]. The effects of ACh on various parameters of the immune system are different and depend on its concentration [1,3,13].

Here we studied the effects of anticholinesterase OPC on AChE in thymic and spleen lymphocytes and evaluated the role of this effect and ACh in modulation of antibody formation.

Saratov State Medical University; Saratov Military Institute of Radiation, Chemical, and Biological Protection

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 180-220 g. Anticholinesterase insecticide dimethyl dichlorovinyl phosphate (DDVP) was injected subcutaneously in a single dose of 0.5 LD_{50} (65±6 mg/kg) 2 days after immunization. ACh was injected subcutaneously in a dose of 5 mg/kg 2 times a day for 3 days 1 day after immunization. Humoral immune response to thymus-dependent antigen (sheep erythrocytes, SE) was evaluated 4 days after intraperitoneal immunization with this antigen in a dose of 2×10^8 cells by the number of antibody-producing cells (APC) in the spleen [8]. These tests reflect IgM synthesis by spleen B cells with the involvement of Th1 lymphocytes. To estimate AChE activity in T cells of the thymus and spleen, T lymphocytes with maximum AChE activity were isolated from nucleated cells in Percoll density gradient (1.065 g/cm^3). The cells were washed with 0.1 M phosphate buffer (pH 8.0), and lymphocytes were counted. These cells constituted 80% low-density T lymphocytes and are characterized by high AChE activity [14]. AChE activity was measured as described elsewhere [6]. ACh iodide (20 μl, 0.075 M) and dithio-bis-nitrobenzoic acid (50 μl, 0.01 M) were added to 1.5 ml suspension containing $1\text{-}5\times10^8$ cells in 1 ml 0.1 M phosphate buffer (pH 8.0).

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Incubation was performed at 25°C for 20 min. The reaction was stopped by adding 100 μl 1,5-difluoro-2,4-dinitrobenzene and optical density was measured at 420 nm [14]. The activity of AChE hydrolyzing 1 μmol ACh in 1 ml suspension containing 10^9 T lymphocytes over 1 min was taken as 1 unit of enzyme activity [10]. Taking into account the fact that antibody formation depends on redistribution of immunocompetent cells between lymphoid organs [11,16], nucleated cells in the spleen and thymus (cell suspension in medium 199) were counted in a Goryaev chamber 4 days after immunization, *i. e.* 1 and 2 days after the last injection of ACh and DDVP, respectively. The number of T and B lymphocytes in the thymus and spleen was estimated [15]. AChE activity was measured as described elsewhere [6,14].

The results were analyzed by Student's *t* test.

RESULTS

T lymphocytes isolated from the thymus and spleen and belonging to low-density T cells with maximum AChE [14] content displayed similar anticholinesterase activity (Table 1).

Administration of ACh increased AChE activity in T cells, which was probably mediated by the mechanisms maintaining immune homeostasis via intensification of ACh hydrolysis on T lymphocyte membranes. The effects of this neurotransmitter on muscarinic and nicotinic receptors on T lymphocytes were thus inhibited [11,12]. These shifts provide a cAMP/cGMP ratio optimum for proliferation and differentiation immunocompetent cells [12]. DDVP markedly inhibited AChE in T cells and decreased the count of APC in the spleen. ACh produced opposite effects (Table 1).

DDVP and ACh decreased the count of T lymphocytes in the thymus, but increased this parameter in the spleen (Table 1).

The decrease in T lymphocyte count in the thymus is related to activation of their muscarinic receptors serving as a signal to cell migration [11]. DDVP decreased the count of B cells in the spleen, which was associated with the effects of corticosteroids (stress reaction) and suppressed proliferation of lymphocytes [4,16]. These changes together with inactivation of AChE in T lymphocytes play a role in suppression of antibody formation [3,16]. DDVP probably reduces the count of T lymphocytes in the spleen, but this effect is compensated by cell migration from the thymus. It is unlikely that ACh stimulates antibody formation via a regulatory reaction, in particular AChE activation in T lymphocytes (Table 1). However, inhibition of this enzyme decreases antibody production. The effects of ACh are related to accumulation of T cells in the spleen and stimulation of their cholinergic receptors, which promote secretion of lymphokines stimulating IgM and IgG_2 synthesis (interleukin-1, interleukin-3, interferon-γ, *etc*.) by Th1 lymphocytes [9]. ACh also potentiates humoral immune response via induction of interferon-γ synthesis in splenocytes and interleukin-1 production by spleen macrophages [1], stimulation of cholinergic receptors on B lymphocytes, *etc*. [3,12,13]. ACh stimulates migration of T cells from the thymus [11] and, therefore, intensifies replacement of thymocytes with suppressed AChE with cells with normal enzyme activity.

TABLE 1. Effects of DDVP (0.5 LD_{50}) and ACh on Functional Activity of the Immune System and Antibody Formation in Wistar Rats 4 Days after Immunization (*M*±*m*, *n*=7-9)

Parameter	Control		ACh		DDVP	
	thymus	spleen	thymus	spleen	thymus	spleen
Cell count, 10^6						
nucleated	975.2±86.7	1433.1±123.6	748.4±69.4*	1556.5±116.9	734.1±64.9*	1400.4±159.3
T lymphocytes	905.3±89.4	703.5±62.3	697.2±46.7*	937.1±90.1*	575.8±44.9*	903.4±65.9*
B lymphocytes	—	633.2±53.6	—	608.0±61.6	—	480.4±54.9*
AChE activity, mU/10^9 cells						
T lymphocytes	68.2±8.1	59.5±8.4	89.6±9.9	79.3±9.3	7.8±2.2*	5.2±1.9*
T lymphocytes isolated from nuclear cells	92.3±9.6	101.7±10.3	120.6±10.2*	133.5±11.1*	11.3±2.8*	9.7±2.5*
Count of antibody-producing cells, 10^3	—	28.2±3.0	—	37.5±3.2*	—	19.4±2.3*

Note. *$p<0.05$ compared to the control.

T cells from the thymus and spleen exhibited similar AChE activity (Table 1). ACh increased AChE activity in T cells by 31-33% ($p>0.05$). B cells possessed no AChE activity. Bearing in mind the existence of T cell subpopulations with high and low AChE contents [14], we used a method allowing us to measure mean AChE activity, which is lower than the total AChE activity measured after cell isolation in Percoll density gradient.

Thus, poisoning with anticholinesterase OPC is accompanied by realization of two opposite effects: inhibition of AChE in T cells leading to suppression of thymus-dependent antibody formation (predominant effect) and ACh-induced stimulation of antibody formation. AChE activation in intact T lymphocytes caused by ACh suggests that AChE in T cell membranes modulates ACh-induced activation of cholinergic receptors, which represent a mechanism regulating the humoral immune response.

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