Combined Effects of Toxicants with Various Mechanisms of Action and Mechanical Trauma on the Immune System

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Experiments on Wistar rats showed that the combined action of mechanical trauma and organophosphorus compound dimethyl dichlorovinyl phosphate a dose of 0.8 LD_{50} was accompanied by summation of their immunosuppressive effects. We observed an additive effect in relation to stimulation of the adrenal cortex. Treatment with general chemical toxicants (acrylic acid nitrile and acetonitrile, 0.8 LD_{50}) alone or in combination with mechanical trauma produced similar increase in plasma corticosterone concentration. This effect was short-lasting and less pronounced compared to the influence of dimethyl dichlorovinyl phosphate. The immunosuppressive effect of trauma and nitriles is primarily related to the influence of poisons.

Key Words: *toxic chemicals; mechanical trauma; combined effects of poisons and trauma; immunosuppression; corticosterone*

The immunotropic effects of poisons that possess anticholinesterase and general toxic activities and cause massive poisoning during accidents at chemical plants [10] were extensively studied [5-7]. Organophosphorus inhibitors of cholinesterase (dimethyl dichlorovinyl phosphate, DDVP) and nitriles (acrylic acid nitrile, AAN; and acetonitrile, AN) belong to toxic chemicals. General toxic effect of nitriles is associated with the influence of their most hazardous metabolite cyanide ion, which causes functional disturbances in enzymes of tissue respiration [7]. Combined effects of mechanical trauma and acute poisoning markedly increase the mortality rate and cause disability in patients. This is related to the development of infectious processes, whose clinical course depends on post-intoxication, post-traumatic [1,9], and immunodeficient state. These situations occur during accidents at plants for the production, storage, and destruction of toxic chemicals [10]. The combined effects of trauma and various poisonings on immune homeostasis remain unclear. Studies of this problem, which is closely related to evaluation of various poststress changes in organisms [11], hold considerable theoretical and practical importance for the therapy and prevention of infectious complications and diseases.

Here we studied the effects of acute poisoning with DDVP, AAN, and AN in combination with severe mechanical trauma on humoral and cellular immune reactions and functional activity of the adrenal cortex.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 180-240 g during daytime (9.00-15.00). In this period plasma corticosterone level in rats is minimum [12]. DDVP, AAN, and AN were injected subcutaneously in a dose of 0.8 LD_{50}. Severe mechanical trauma was produced as described elsewhere [1]. When we assayed the combined action of these factors, acute poisoning was produced 5 min after the incidence of trauma.

Immunization was performed 5-20 min after individual or combined effect of these factors. The humoral immune response was evaluated 5 days after intraperitoneal immunization with thymus-dependent

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TABLE 1. Combined Action of Trauma and Acute Poisoning on Humoral Immune Response (*M*±*m*)

Treatment	Titer of antibodies to sheep erythrocytes, $-\log_2$ titer	Antibody-producing cells, $\times 10^3$	
		against sheep erythrocytes	against Vi-Ag
Control	5.8±0.1 (30)	28.4±1.2 (30)	21.8±1.1 (30)
Trauma	4.5±0.4* (6)	16.3±2.8* (6)	13.0±2.2* (7)
DDVP	3.6±0.3* (7)	14.1±2.7* (7)	15.8±2.6* (7)
DDVP+trauma	2.3±0.3° (8)	6.7±1.7° (8)	9.1±2.3* (7)
AAN	3.7+0.3* (6)	13.1±2.4* (6)	14.3±2.5* (6)
AAN+trauma	3.5±0.2* (6)	14.2±2.1* (7)	13.9±2.1* (8)
AN	3.3±0.4* (6)	13.7±2.3* (6)	16.0±2.2* (6)
AN+trauma	3.0±0.2* (5)	12.5±2.2* (7)	12.7±1.9* (8)

Note. Here and in Tables 2 and 3: number of animals is shown in brackets. $p<0.05$: *compared to the control; °compared to the control and influence of trauma or toxic chemicals alone.

(sheep erythrocytes) and thymus-independent antigens (Vi-Ag) in doses of 2×10^8 cells and 8 μg/kg, respectively, by the number of antibody-producing cells in the spleen [4,14]. The humoral immune response to sheep erythrocytes characterizes the involvement of Th1 lymphocytes in IgM production by B lymphocytes (plasma cells).

Activity of natural killer cells was estimated by natural cytotoxicity 48 h after combined action of the studied factors [3]. Antibody-dependent cytotoxicity was assayed spectrophotometrically 5 days after immunization with sheep erythrocytes (10^8 cells) [8]. To study the cellular immune response (*e.g.*, Th1 lymphocyte activity), the rats were intraperitoneally immunized with sheep erythrocytes (10^8 cells). Sheep erythrocytes in a provoking dose (5×10^8 cells) were administered subaponeurotically into hindlimb pads 4 days later. Delayed-type hypersensitivity was evaluated by the increase in hindpaw weight 24 h postinjection.

The count of T cells in the thymus was estimated routinely 24 h after treatment. The number of nucleated cells was evaluated taking into account the fact that lymphocytes in the thymus are mainly presented by T lymphocytes. To assay functional activity of the adrenal cortex, corticosterone concentration was measured fluorometrically 1, 3, and 24 h after treatment [4]. The results were analyzed by Student's *t* test.

RESULTS

Severe mechanical trauma and acute poisoning with toxic chemicals were followed by a significant decrease in the negative binary logarithm of anti-sheep erythrocyte antibody titer (Table 1). The count of spleen cells producing antibodies against sheep erythrocytes and Vi-Ag underwent similar changes.

Summation of the effects was observed only after combined action of DDVP and trauma. During combined action of trauma and nitriles the immunosuppressive effects were produced only by poisons. After mechanical trauma, treatment with toxic chemicals, or combined action of these factors, the count of cells producing antibodies against T cell-independent Vi-Ag decreased to a lesser degree compared to the num-

TABLE 2. Combined Action of Trauma and Acute Poisoning on Cellular Immune Response (*M*±*m*)

Treatment	T cell count in the thymus, 10^6	Natural cytotoxicity, %	Antibody-dependent cell cytotoxicity, %	Delayed-type hypersensitivity response, increase in paw weight, %
Control	762±44 (25)	31.5±1.2 (27)	11.8±0.5 (25)	29.5±0.8 (25)
Trauma	441±51* (6)	17.2±2.2* (11)	6.9±1.4* (9)	17.2±1.4* (9)
DDVP	487±67* (10)	12.4±2.1* (8)	5.8±1.0* (10)	15.1±1.5* (8)
DDVP+trauma	312±43° (10)	8.1±1.3** (9)	3.7±0.8** (10)	10.5±1.1° (9)
AAN	579±53* (7)	17.0±2.7* (9)	6.3±1.1* (7)	14.8±1.2* (10)
AAN+trauma	438±45* (7)	16.1±2.4* (9)	6.1±1.3* (7)	13.1±1.3* (10)
AN	525±57 *(10)	15.0±2.5* (10)	7.0±1.2* (8)	11.9±1.6* (7)
AN+trauma	450±39* (10)	14.5±1.3* (10)	8.2±1.5* (8)	12.6±1.7* (7)

TABLE 3. Combined Action of Trauma and Acute Poisoning on Plasma Concentration of Corticosterone ($M \pm m$, n=7-9)

Treatment	Control	Time after treatment, h		
		1	3	24
Trauma	17.1±1.6	51.6±5.5*	48.9±3.7*	19.5±2.2
DDVP	15.8±1.9	75.8±6.3*	43.4±4.1*	21.8±2.7**
DDVP+trauma	16.5±2.1	130.7±11.2°	90.5±7.5°	22.7±2.5**
AAN	20.2±2.8	30.1±2.5*	15.5±1.5	12.1±2.1*
AAN+trauma	16.9±3.0	31.5±5.0*	21.1±2.4	10.3±2.4*
AN	21.0±2.3	29.5±3.0*	17.9±1.6	11.7±2.7*
AN+trauma	18.3±3.1	32.0±5.2*	25.7±2.8	13.2±2.0*

Note. **p<0.05 compared to the effects of AAN and AN alone or in combination with trauma.

ber of cells synthesizing antibodies to sheep erythrocytes.

Severe mechanical trauma and acute poisoning decreased T cell count in the thymus, reduced activity of natural killer cells, and suppressed the delayed-type hypersensitivity response (Table 2). Summation of the immunosuppressive effects was observed after combined action of DDVP and trauma. Suppression of cell immune reactions after combined action of general toxicants and trauma depended on the type of toxicants.

Plasma corticosterone concentration increased by 3.0 and 4.8 times 1 h after trauma and administration of DDVP, respectively; 3 h after treatment this parameter increased by 2.8 and 2.7 times, respectively (Table 3). One hour after acute poisoning with AAN and AN plasma corticosterone level increased by 1.5 and 1.4 times, respectively, which was probably associated with the post-intoxication stress reaction. Suppression of corticosterone synthesis 3 and 24 h after treatment was due to inhibition of a_3 component in cytochrome *c* oxidase (mitochondrial respiratory enzyme in the adrenal cortex) by cyanide ion, the main metabolite of nitriles [13]. As differentiated from the combined action of trauma and general toxicants, summation of the effects was observed 1 and 3 h after the exposure to DDVP and traumatic injury. Plasma corticosterone concentration 24 h after treatment with DDVP alone or in combination with trauma was much higher than after individual or combined action of nitriles and traumatic injury.

These data demonstrate the relationship between plasma corticosterone level and immunosuppressive effect of DDVP and trauma. Summation of the effects was observed after combined action of poisoning with this organophosphorus compound and trauma. The immunotoxic effect after acute poisoning with DDVP is determined by not only increased concentration of corticosterone, but also other factors [5]. Suppression of the immune system induced by nitriles alone or in combination with mechanical trauma is not related to the effect of corticosterone [7].

Our results show that after combined action of chemical toxicants and trauma, changes in the immune system depend on immunotropic properties of these agents and post-traumatic dysfunction of the adrenal cortex.

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