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EFFECT OF AN ANTIOXIDANT INHIBITOR OF THE 3-HYDROXYPYRIDINE SERIES ON THE CELL-MEDIATE IMMUNE RESPONSE

A. A. Suzdal'tseva, M. M. Kapichnikov, Yu. A. Shalyminova, Yu. V. Zykov, and L. D. Smirnov

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In connection with the search for effective immunoregulators the study of antioxidants which affect the immune response is very important [5]. We know that administration of natural (vitamin E) or synthetic (ionol, santoquin) antioxidants is known to stimulate humoral and cellular immunity in experimental animals [6], whereas antioxidants belonging to the β-hydroxy derivatives of heterocyclic compounds containing nitrogen exhibit marked immunodepressive activity [3, 4].

The writers previously discovered the immunodepressive action of an antioxidant inhibitor belonging to the 3-hydroxypyridine class (preparation OP-6) on a model of synthesis of antibodies against sheep's red blood cells, and also during allografting of the skin in mice [1, 3].

The object of the present investigation was to continue the study of the immunodepressive activity of compound OP-6, using methods of simultaneous evaluation of mitostatic and lymphotoxic action [2] and interaction between sensitized lymphocytes and target cells [7].

TABLE 1. Mitostatic and Lymphotoxic Activity of Compound OP-6 on Sublethally Irradiated Mice

Dose of compound, mg/kg	Mean No. of CFU per spleen after admin. of comp. (M ± m)	Mitostatic effect	Mean No. of CFU per spleen after injection of compound and $2 \cdot 10^6$ lymphocytes (M ± m)	No. of surviving CFU (in %) – lymphotoxic effect
–	12,1±1,3	–	4,4±0,9	36,7
50	12,5±0,9	Absent	9,2±0,8	66,6
100	12,2±1,2	"	8,1±0,6	72,6
150	11,8±1,9	"	10,7±0,8	88,5
200	12,1±1,2	"	11,6±0,9	96,1

Department of Operative Surgery and Topographic Anatomy, Clinical Center for Andrology and Transplantation of Endocrine Glands, P. Lumumba Peoples' Friendship University. Laboratory of Histocompatibility, Research Institute of Immunology, Academy of Medical Sciences of the USSR, Moscow. [Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov (deceased).] Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 2, pp. 49-50, February, 1982. Original article submitted May 8, 1981.

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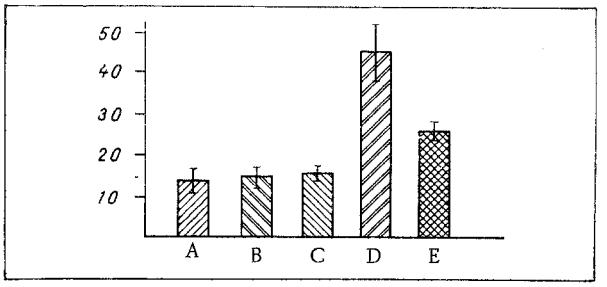

Fig. 1. Effect of compound OP-6 in a dose of 100 mg/kg on interaction of intact and sensitized lymphocytes with target cells. A) Spontaneous death of target cells; B) death of target cells following interaction with lymphocytes of intact mice; C) death of target cells following interaction with lymphocytes of intact mice treated with compound OP-6; D) death of target cells during interaction with lymphocytes of sensitized mice; E) death of target cells following interaction with lymphocytes of sensitized mice treated with compound OP-6. Ordinate, mortality of target cells, %.

EXPERIMENTAL METHOD

To determine the mitostatic and lymphotoxic action of the compound, (CBA × C57BL)F_1 mice weighing 18-20 g were subjected to sublethal irradiation in a dose of 600 R on a Stebel' 3A apparatus. After 24 h the animals were given a single intraperitoneal injection of compound OP-6 in doses of 50, 100, 150, and 200 mg/kg (10 groups, with 20 mice in each group). After an interval of 1 h $2 \cdot 10^6$ lymph node cells from mice of the parental CBA line were injected intravenously into half of the mice.

Nine days later the animals were killed, their spleens were removed and, after fixation, the number of endogenous colony-forming units (CFU) in them was counted.

To study the immunodepressive action of compound OP-6 on cytotoxic activity of lymphocytes, 2-day cultures of embryonic fibroblasts from C57BL/6j mice were used as target cells. The effector cells were lymphocytes obtained from the spleens of CBA mice on the 7th day after a single intraperitoneal immunization with lymphoid cells from C57BL/6j mice in a dose of $5 \cdot 10^7$ cells. Lymphocytes of intact CBA mice were used in the control.

Compound OP-6, in a dose of 100 mg/kg, was injected into mice of two groups: It was injected intraperitoneally daily for 3 days into intact and sensitized mice (for 3 days altogether, intraperitoneally, starting with the day of immunization).

The cytotoxic effect was determined 24 h after combined incubation of the cells, in the ratio of 100 lymphocytes per target cell, by counting the number of dead target cells and calculating their percentage in the experiment and control.

The numerical results were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

The study of the mitostatic action of compound OP-6 showed that its injection in doses of 50, 100, 150, and 200 mg/kg into sublethally irradiated mice had no visible effect on endogenous colony formation (Table 1). Consequently, compound OP-6, in all doses tested, did not inhibit proliferation of hematopoietic stem cells, i.e., it had no mitostatic action.

In addition, compound OP-6 was found to have a marked lymphotoxic action, in the same doses, on immunocompetent lymphoid cells.

As Table 1 shows, intravenous injection of lymph node cells of the parental genotype into sublethally irradiated (CBA × C57BL)F_1 mice caused marked depression of endogenous colony

formation: only 36.7% of CFU survived compared with 100% in the irradiation control. Preliminary treatment of these mice with compound OP-6 completely or largely abolished this inhibition; attention must be paid to the well-marked dose dependence of the lymphocytotoxic action of the compound (Table 1). Differences between the control and experimental groups in all cases were highly significant ($P < 0.001$).

The use of the method of simultaneous evaluation of mitostatic and lymphotoxic action thus enabled the different effects of compound OP-6 on stem and lymphoid cells to be differentiated.

The lymphocytotoxic effect of compound OP-6 also was clearly demonstrated on a model of interaction between sensitized lymphocytes and target cells (Fig. 1).

As Fig. 1 shows, culture of fibroblasts without lymphocytes was followed by spontaneous death of 12.6 ± 3.1% of the target cells. Addition of allogeneic lymphocytes from intact mice to the culture of fibroblasts caused death of 14.3 ± 2.2% of the target cells, evidence that the effector cells of intact mice have no cytotoxic action. Injection of compound OP-6 into intact animals also had practically no effect on interaction between lymphocytes obtained from them and fibroblasts: the number of dead target cells was 14.8 ± 1.2%, i.e., it was also at the control level.

However, the number of dead fibroblasts was considerably increased after their interaction with lymphocytes obtained from specifically sensitized mice, when death of 44.5 ± 5.8% of fibroblasts was observed. In other words, specifically sensitized allogeneic lymphocytes exhibited marked cytotoxic activity against target cells. Injection of compound OP-6 into immunized mice acting as donors of effector cells was followed by a highly significant ($P < 0.001$) reduction in the cytotoxic activity of lymphocytes obtained from these mice: The number of dying target cells fell to 25.6 ± 2.7%.

These results are thus evidence that an antioxidant inhibitor (compound OP-6) depresses the cytotoxic function of T killer cells without exerting any appreciable inhibitory effect on the proliferative ability of hematopoietic stem cells. These properties which compound OP-6 was found to possess justify its inclusion among the immunodepressants with direct action.

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