EFFECT OF AN ANTIOXIDANT INHIBITOR OF THE 3-HYDROXY-PYRIDINE CLASS ON IMMUNOCOMPETENT AND STEM CELLS

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The effect of a synthetic antioxidant inhibitor belonging to the 3-hydroxypyridine class, compound OP-6, on functions of immunocompetent and stem cells was studied in mice. Models of synthesis of antibodies against sheep's red blood cells (local hemolysis and hemagglutination tests), endogenous and exogenous colony formation in (CBA × C57BL/6j)F_1 mice and homografting CBA mice with skin from C57BL/6j donors were used. Compound OP-6 was shown to have an immunodepressive action on both humoral and cellular immunity, but it did not depress the colony-forming ability of the stem cells or protect them against the action of irradiation.

KEY WORDS: transplantation immunity; antibody formation; colony formation; immunodepression.

Natural and synthetic antioxidant inhibitors (vitamin E, alkyl-substituted phenols, and 3-hydroxypyridines) have recently found increasingly wide application in biology and medicine [4, 5]. They have antitumor and radioprotective properties as well as other types of biological activity [2]. These properties of the antioxidant inhibitors may perhaps be connected with their effect on the intensity of free-radical reactions in various components of the cell and also with their action on the physicochemical properties and functional activity of membranes [1].

It was this last effect which motivated the present study of the immunotropic properties of antioxidant inhibitors, for a connection has been shown between the immunodepressive action of a number of pharmacological agents and their effects on permeability of lymphocyte plasma membranes [3]. In the investigation described below the effect of compound OP-6, a synthetic antioxidant inhibitor belonging to the 3-hydroxypyridine class, on functions of immunocompetent and stem cells was studied in mice.

EXPERIMENTAL METHOD

Synthesis of antibodies against sheep's red blood cells (SRBC) was studied by Jerne's local hemolysis test [6] and by the hemagglutination test, endogenous and exogenous colony formation was studied by the method of Till and McCulloch [7], and survival of skin grafts from C57BL/6j donors transplanted on CBA mice were studied in experiments on (CBA × C57BL/6j)F_1 mice.

Water-soluble compound OP-6 was injected intraperitoneally into mice (three series of experiments, 15 mice in each series) in single doses of 50, 100, and 200 mg/kg respectively 24 h before, on the day of, and 24 h after immunization with 0.5 ml of a 10% suspension of SRBC. The compound was injected into mice of a separate group in a dose of 100 mg/kg simultaneously with immunization, and thereafter for 4 days. On the 5th day after immunization the antibody titer was determined in the blood serum by the direct hemagglutination test. The number of antibody-forming cells (AFC) per 10×10^6 spleen cells was counted by the local hemolysis test.

To produce endogenous colony formation the mice were irradiated on the RUM-200 apparatus at the rate of 41 R/min in doses of 600, 700, 800, and 900 R. Compound OP-6 was injected in a single dose of 200 mg/kg into mice of three groups (30 animals in each group) 30 min before irradiation.

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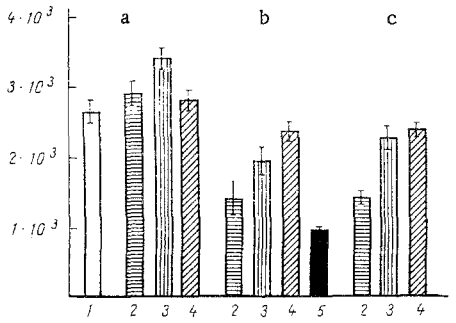

Fig. 1. Action of compound OP-6 on synthesis of antibodies against SRBC in mouse spleen depending on dose and time of injection. Ordinate, number of AFC per 10×10^6 spleen cells. a) Compound OP-6 injected 24 h before immunization, b) on day of immunization, c) 24 h after immunization. 1) Control; 2) 200 mg/kg once only; 3) 100 mg/kg once only; 4) 50 mg/kg once only; 5) 100 mg/kg daily for 5 days.

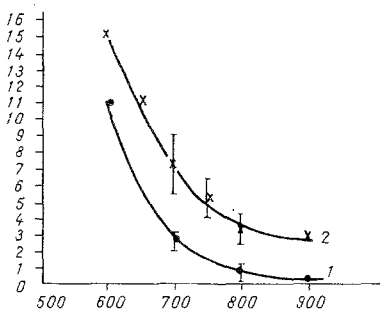

Fig. 2. Effect of compound OP-6 on colony formation in mouse spleen after different doses of irradiation. Abscissa, dose of irradiation (in R); ordinate, number of colony-forming units per spleen. 1) Control, 2) experiment.

Exogenous colonies in the spleen were counted in mice irradiated in a dose of 1200 rad from a ^{137}Cs source of γ rays. Intraperitoneal injections of 10^5 spleen cells were given to 30 mice 24 h later, another 30 mice received 10^5 bone marrow cells intravenously, and 30 mice served as controls. Compound OP-6 was injected as a single dose of 200 mg/kg after 4-6 h.

Skin grafts measuring 1.5×1.5 cm were transplanted on the dorsal region in the usual way. Compound OP-6 was injected intraperitoneally, starting 2 days before or on the day of grafting, in a dose of 100 mg/kg daily until the first signs of rejection appeared. The mean length of survival of the grafts served as a measure of its action.

The results of all the experiments were subjected to statistical analysis by the Fisher–Student method.

EXPERIMENTAL RESULTS

The local hemolysis in gel test revealed a decrease in the number of AFC in the spleens of the mice following a single injection of compound OP-6 in a dose of 200 mg/kg simultaneously with immunization. An even greater decrease in the number of AFC was observed when compound OP-6 was given simultaneously with and for the next 4 days after immunization in a daily dose of 100 mg/kg (Fig. 1). Differences from the

TABLE 1. Length of Survival of Skin Allografts on Mice Treated with Compound OP-6

Dose of compound OP-6, mg/kg	Beginning of administration of compound	Length of survival of graft (M±m)	P
100	Two days before grafting	11,4±0,59	0,01
100	On day of grafting	13,2±0,75	0,001
Control	—	8,4±0,5	—

control were statistically significant in both groups (P = 0.001). Hemagglutinin titers also fell from 1:32-1:64 to 1:4-1:8.

A single injection of the compound into the animals in doses of 100 and 50 mg/kg simultaneously with immunization had no immunodepressive action. When the compound was injected in a single dose 24 h before immunization, no decrease in the number of AFC was observed whatever the dose given; conversely, a dose of 100 mg/kg at this time had a slight stimulating action (Fig. 1). Differences from the control are statistically significant (P = 0.004). Injection of the compound into the animals in doses of 50 and 100 mg/kg 24 h after immunization with SRBC was ineffective. A dose of 200 mg/kg reduced the number of plaques (P < 0.001).

The results are evidence that compound OP-6 has an immunodepressive action when injected as a single dose of 200 mg/kg simultaneously with or 24 h after immunization, and also when injected repeatedly (5 times) in a dose of 100 mg/kg, starting from the day of immunization. Injection of the compound in a dose of 100 mg/kg 24 h before immunization with SRBC, however, had a stimulating action on antibody synthesis.

The study of the kinetics of colony formation in the spleen following injection of compound OP-6 showed a delayed decrease in the number of endogenous colony-forming units (CFU) compared with the control, depending on the dose of irradiation (Fig. 2). The results are evidence of the protective action of the compound on stem cells.

On a model of exogenous colony formation, in which 10^5 bone marrow cells were transplanted from syngeneic donors, compound OP-6 did not depress the CFU (12.4 ± 1.2 in the experiment, 10.4 ± 1.1 in the control). After injection of 10^5 spleen cells under these same conditions there was a very slight tendency for colony formation to be inhibited. In the experiment in which compound OP-6 was used the number of CFU was 3.4 ± 0.5, compared with 5.0 ± 0.8 in the control. These results show that a single injection of the compound into the recipient had no marked effect on colony formation. Meanwhile, administration of compound OP-6 to the bone marrow donors in a single dose of 200 mg/kg 4-6 h before transplantation of the marrow into recipient mice gave an increase in the number of exogenous colonies in the spleen to 14.2 ± 0.43 compared with 9.5 ± 0.45 in the control after injection of bone marrow of intact donors (differences significant, P < 0.01).

These results suggest that the compound has no harmful effect on stem cells, a conclusion which is also confirmed by the writers' previous results showing the radioprotective action of OP-6 on bone marrow cells.

After skin grafting, compound OP-6 lengthened the average survival time of the grafts (Table 1).

Consequently, compound OP-6 has an immunodepressive action on both the humoral and the cellular components of the immune response. The results obtained on several different models are evidence that compound OP-6, with its immunodepressive properties, selectively depresses immunocompetent cells but causes virtually no decrease in the colony-forming ability of the recipient's hematopoietic stem cells or of the donor's cells growing in the recipient, and protects them against the effects of irradiation.

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