Radiometric Evaluation of the Effect of Xenobiotics on Proliferation of Bone Marrow Cells in Mice

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A radiometric method of evaluating the effect of chemical compounds on the proliferative activity of bone marrow cells is described which consists of measuring the incorporation of labeled ^{3}H-thymidine in DNA. Results are reported on a comparative study of the effect of known immunotropic substances on bone marrow cell proliferation using the present method and the method of evaluating endogenous colony formation. Analysis of the results obtained by two variants of *in vivo* and *in vitro* experiments provides additional information regarding the mechanism of action of the substances.

Key Words: *immunomodulators; bone marrow; endogenous colony formation*

In order to evaluate the immunomodulating and immunotoxic properties of pharmaceutical agents, besides studying their effect on the T and B systems of immunity and the system of mononuclear phagocytes, researchers also assay their effect on bone marrow cells (BMC) [4,5]. The bone marrow is a key element of the immune system, containing polypotent stem cells which are thought to be precursors of all the blood cells, including all the subpopulations of lymphocytes. Therefore, the damaging or modulating effect of chemical compounds has an impact on the immune response of the organism. BMC are one of the most rapidly proliferating cell populations, and as a consequence they are especially sensitive to various toxic agents. This makes them a very convenient model for the evaluation of the mitostatic effect of xenobiotics. The functional status of BMC is usually determined by their capacity for colony formation either *in vivo* in the spleen of sublethally irradiated animals or *in vitro* in semiliquid matrixes with appropriate mitotic stimulation [3,4]. Both methods are quite laborious and time-consuming, and the first one requires sophisticated irradiation equipment. We propose a simple and convenient method of assessing the effect of chemical compounds on BMC proliferative activity according to the incorporation of labeled ^{3}H-thymidine.

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MATERIALS AND METHODS

Male mice (CBA×C57Bl) F_1 weighing 20-22 g were used for the experiments. CBA, C57Bl/6, and BALB/c mice, as well as nonpure-strain mice were used in some cases. The animals were obtained from the Stolbovaya breeding center and kept at 20-22°C in a 12-h light regime. The mice were irradiated with an EKU-6 apparatus (dose 500 R) to determine the effect of agents on the level of endogenous colony formation. The preparations were administered intraperitoneally according to the following scheme: -1, 0, 1, and 2, where 0 is the day of irradiation. The animals were killed on the 8th day of irradiation by means of cervical dislocation, the spleens were removed, and endogenous colonies were counted.

For evaluation of the *in vivo* effect on BMC proliferation the preparations were administered

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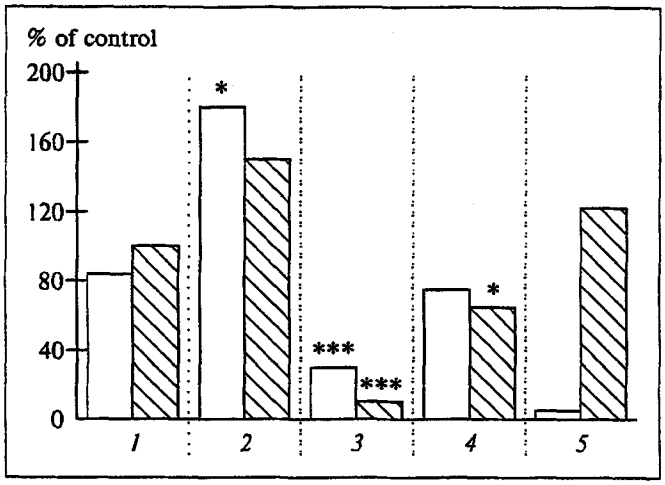

Fig. 1. Effect of test preparations on endogenous colony formation (light bar) and ^{3}H-thymidine incorporation (dark bar): *1*) levamisole, 2.5 mg/kg; *2*) methyluracil, 100 mg/kg; *3*) cyclophosphane, 50 mg/kg; *4*) Tween-80, 5 mg/kg; *5*) sarcolysine, 5 mg/kg.

intraperitoneally according to the scheme: -3, -2, and -1 (where 0 is the day of sacrifice). BMC were isolated routinely from the femur [2], and resuspended in RPMI-1640 medium containing 10% fetal calf serum and antibiotics: penicillin (100 IU/ml) and streptomycin (100 μg/ml).

Cell suspension (170 μl) was introduced into 96-well round-bottom plates for immunological reactions (Medpolymer), supplemented with 1 μCi ^{3}H-thymidine, and incubated for 4 h at 37°C in a humidified atmosphere with 5% CO_2. Levels of label incorporation were determined individually for each mouse in 5 replications.

In vitro experiments were performed where BMC of intact mice were incubated for 24 h in culture medium with preparations added in concentrations of 1, 10 and 100 μg/ml. ^{3}H-thymidine was then added. After 16 h, cell suspension (75 μl) was applied to FN-8 filter papers (Filtrak) (2×2 cm), dried, rinsed twice with 0.15 M NaCl for 5 min, held for 24 h at 4°C in 5% trichloroacetic acid, rinsed twice for 5 min, and dried [1]. Radioactivity was measured in ZhS-8 scintillation liquid (Reakhim) on a Beta-1 counter (Pilot Plant, Research Institute of Medical Instrumentation).

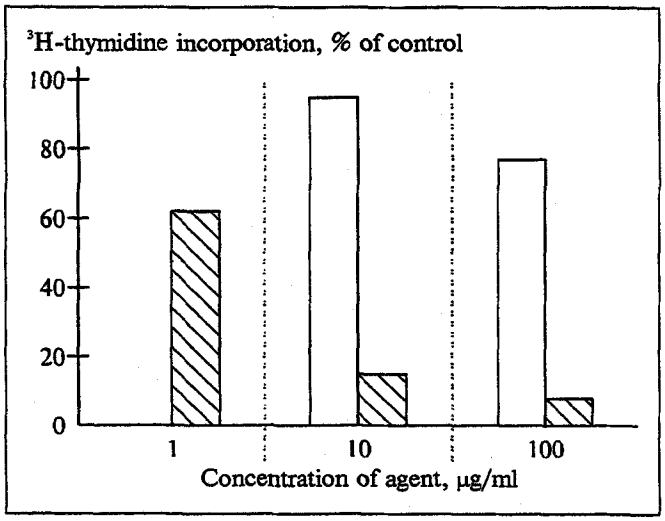

Fig. 2. Effect of cyclophosphane (light bars) and sarcolysine (dark bar) on BMC proliferation *in vitro*.

RESULTS

Two modifications were developed to evaluate the effect of pharmacological agents on BMC proliferative activity: 1) the preparations were administered to animals and then label incorporation by BMC was determined; 2) BMC were isolated from intact animals and label incorporation was determined after incubation with the agent.

The second variant proved to be more economical; however, the first one is preferable, as it provides a possibility for metabolic activation of preparations. Special preliminary experiments established that the experimental conditions for evaluating ^{3}H-thymidine incorporation (as described in Material and Methods) are optimal.

We compared the effect of levamisole (2.5 mg/kg), methyluracil (100 mg/kg), cyclophosphane (50 mg/kg), sarcolysine (5 mg/kg), and 0.1% Tween-80 solution on the level of endogenous colony formation in irradiated mice and the level of BMC proliferative activity during *in vivo* administration of the preparations (Fig. 1).

Correlation analysis was performed. Figure 1 shows that the level of ^{3}H-thymidine incorporation correlates with the number of endogenous colonies (correlation coefficient r=0.98 without the data on sarcolysine being taken into account).

The results obtained by both methods are consistent with the pharmacological activity of the agents. The cytostatic cyclophosphane and Tween-80, which, according to our findings, is able to lower immune reactions, inhibited BMC proliferation and colony formation. Methyluracil, an immunostimulator, enhanced these parameters, whereas levamisole was found to be inactive in both tests.

Sarcolysine was the only exception (Fig. 1). It completely inhibited endogenous colony formation; however, it not only did not inhibit incorporation of the label, but even somewhat stimulated it. The effect of sarcolysine is probably associated with its specific pharmacokinetics. Sarcolysine evidently does not penetrate BMC and is active only in respect to those precursor cells which have entered the bloodstream and spleen. In fact, *in vitro* incu-

bation of BMC with sarcolysine showed a sharp dose-dependent inhibition of proliferation evaluated by the level of ^{3}H-thymidine incorporation (Fig. 2). Figure 2 presents for comparison a graph of ^{3}H-thymidine incorporation into BMC preliminarily preincubated with cyclophosphane *in vitro*. Cyclophosphane is seen to have a weak effect on cell proliferation *in vitro*, whereas it does have a cytostatic effect *in vivo* (Fig. 1). This is because metabolic activity is needed for cyclophosphane to manifest an effect and this is impossible under *in vitro* conditions.

Thus, the radiometric method proposed here is suitable for evaluating the effect of chemical compounds on BMC proliferation, takes little time, and does not require sophisticated irradiation equipment; moreover, comparative analysis of *in vitro* and *in vivo* results provides additional information on the mechanism of action of the agents.

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