EFFECT OF VINBLASTIN ON COLONY FORMATION IN MONOLAYER BONE MARROW CULTURES AND ON HEMATOPOIESIS IN GUINEA PIGS

G. D. Baisogolov, S. F. Rudakova,
I. A. Rudakov, and A. G. Konoplyannikov

UDC 612.419.014.46.085.2

The effect of vinblastin (0.15-2 mg/kg, intraperitoneally) on hematopoiesis and development of colonies of fibroblasts in monolayer cultures of the guinea pig bone marrow was studied. After injection of vinblastin the total number of nucleated cells in the femur was reduced while the concentration of colony-forming cells increased. Correlation probably exists between the precursor cells of fibroblasts in monolayer cultures of hematopoietic tissue and hematopoietic stem cells.

The study of the nature of the cells forming discrete colonies of fibroblast-like cells in monolayer cultures of hematopoietic tissue and the elucidation of the relationship of these cells to the hematopoietic stem cells are problems requiring urgent solution. A promising line of research in this respect is the investigation of the response of colony-forming cells to the action of extreme stimuli with a marked effect on hematopoiesis. The writers showed previously that injection of vinblastin is followed by an increase in the yield of endogenous colonies in irradiated mice [1].

In the present investigation the effect of vinblastin was studied on the colony-forming cells of the guinea pig bone marrow in vitro.

EXPERIMENTAL METHOD

Experiments were carried out on guinea pigs of both sexes weighing 260-280 g. The colony-forming ability of the bone marrow in monolayer cultures [4] and the indices of hematopoiesis were studied during the first 4 days after intraperitoneal injection of vinblastin in doses of 0.15, 0.5, and 2 mg/kg. Cells for explantation were obtained from the central zone of the femoral marrow. A suspension of cells from three to five animals in medium No. 199 was added to 250-ml flasks for cultivation. The culture medium consisted of 80% medium No. 199 and 20% bovine serum with the addition of 50 units penicillin and streptomycin per ml. The cultures were fixed with absolute alcohol on the 10th-11th day and stained with freshly made hematoxylin. The colonies in the flasks were counted under a type MBS-1 binocular loupe. Indices of the blood and bone marrow were investigated in all experimental animals. Altogether 75 guinea pigs were used in the experiments and 120 cultures were set up.

EXPERIMENTAL RESULTS

Injection of vinblastin had a marked action on the hematopoietic indices of the animals. The number of bone marrow cells fell after all doses of the preparation, and the decrease was most marked after injection of vinblastin in a dose of 2 mg/kg (Fig. 1). With this dose some animals died during the first 4 days after injection of the preparation. After a dose of 0.5 mg/kg the number of myelokaryocytes also fell significantly, although by a lesser degree. In a dose of 0.15 mg/kg the total number of myelokaryocytes fell by

Research Institute of Medical Radiology of the USSR, Obninsk. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 9, pp. 95-98, September, 1972. Original article submitted January 31, 1972.

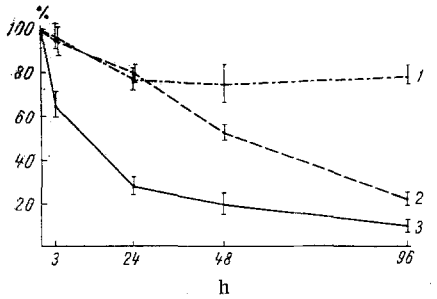

Fig. 1

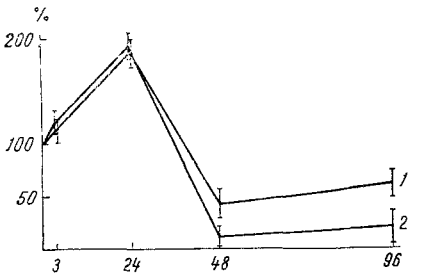

Fig. 2

Fig. 1. Number of myelokaryocytes in bone marrow of guinea pigs after injection of vinblastin in doses of 0.15 (1), 0.5 (2), and 2 (3) mg/kg. Abscissa, time (in h) after injection of vinblastin; ordinate, number of myelokaryocytes (in percent of initial value).

Fig. 2. Number of leukocytes in blood of guinea pigs after injection of vinblastin in doses of 0.15 (1) and 2 (2) mg/kg. Abscissa, time (in hours) after injection of vinblastin; ordinate, leukocyte count (in percent of initial level).

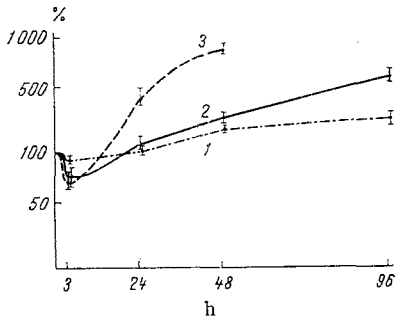

Fig. 3. Number of colony-forming cells in bone marrow of guinea pigs after injection of vinblastin in doses of 0.15 (1), 0.5 (2), and 2 (3) mg/kg. Abscissa, time (in hours); ordinate, number of colony-forming cells (in percent of initial level).

20% during the 1st day, but thereafter showed no further decrease. A decrease in the number of lymphocytes and of immature cells of the granulocytic and erythroid series was observed in the myelogram of the animals starting from the 3rd hour after injection of vinblastin. Throughout the period of observation the erythrocyte count and hemoglobin concentration in the peripheral blood remained substantially unchanged, although the leukocyte count varied significantly (Fig. 2). The effectiveness of colony formation in the bone marrow of the intact guinea pigs varied from 15 to 20 colonies per 10^6 explanted cells. After injection of vinblastin the initial transient decrease in the number of colony-forming cells in the bone marrow was followed by recovery and by an increase in their number (Fig. 3). The higher the dose of vinblastin, the more marked the increase in the relative number of these cells in the bone marrow. Despite the fact that the increase in the number of colony-forming cells in the bone marrow was observed against the background of a decrease in the total number of cells, there was also some increase in the absolute number of colony-forming cells calculated for the bone marrow of the whole bone.

It appeared useful to compare these results with those for hematopoietic precursor cells obtained by other methods. The harmful action of vinblastin on hematopoiesis is considered to be connected with the arrest of division at the metaphase stage with subsequent lysis of the cells [6]. In mice which received an injection of vinblastin 2 h before irradiation, an increase was observed in the yield of endogenous colonies and better restoration of the number of bone marrow cells and the other indices of hematopoiesis were observed [5]. The present experiment showed that the increase in number of endogenous colonies in the mice irradiated 1-4 days after injection of vinblastin was accompanied by an increase in the radiosensitivity of the colony-forming cells [1]. The dynamics of the colony-forming cells in monolayer cultures of the guinea pig bone marrow after injection of vinblastin were very similar on the whole to the changes in the number of cells responsible for the formation of hematopoietic colonies in the spleen of the irradiated mice: after a transient decrease in the number of cells of this fraction lasting a few hours, recovery took place and their number in the bone marrow increased. The dynamics of the change in the number of colony-forming cells and in the total number of bone marrow cells as a whole was completely different.

The results for the action of vinblastin on the colony-forming ability of bone marrow cells in monolayer cultures, combined with reactions of these cells to blood loss, antithrombocytic serum, and external

radiation studied previously [2, 3], suggest that cells forming discrete colonies of fibroblast-like cells in monolayer cultures of hematopoietic tissue belong to the population of early precursors of hematopoietic cells; as a result of a study of their dynamics it is possible to judge to some extent the state of the deep reserves of hematopoiesis, a very important factor in connection with the possible use of the monolayer culture method in various diseases or in the course of treatment accompanied by damage to the hematopoietic system.

LITERATURE CITED

1. G. D. Baisogolov, A. G. Konoplyannikov, A. M. Poverennyi, et al., in: Problems in Radiobiology and the Biological Action of Cytostatic Preparations [in Russian], Tomsk (1969), p. 74.
2. A. G. Konoplyannikov, S. F. Rudakova, I. A. Rudakov, et al., in: Proceedings of an All-Union Symposium on Problems of the Conservation, Cultivation, and Typing of Bone Marrow [in Russian], Moscow (1971), p. 110.
3. I. A. Rudakov, S. F. Rudakova, R. L. Maslennikova, et al., in: Proceedings of an All-Union Symposium on Problems of the Conservation, Cultivation, and Typing of Bone Marrow [in Russian], Moscow (1971), p. 113.
4. A. Ya. Fridenshtein, E. A. Luriya, R. K. Chailakhyan, et al., Vestn. Akad. Med. Nauk SSSR, No. 7, 86 (1970).
5. W. Smith and S. Wilson, J. Nat. Cancer Inst., 39, 1055 (1967).
6. F. A. Valeriote and W. R. Bruce, J. Nat. Cancer Inst., 39, 393 (1967).