ACTION OF IMMUNOMODULATORS AND CYTOSTATICS ON ^{125}I-DEOXYURIDINE CATABOLISM IN TUMORS (RAPID SCREENING OF ANTITUMOR IMMUNOMODULATORS)

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UDC 615.277.3.015.4:616-006-008.9].076.9

KEY WORDS: immunomodulators; cytostatics; deoxyuridine; tumor

The effect of antitumor preparations and immunomodulators in vivo is generally judged by two principal parameters: delayed growth and reduced size or total disappearance of tumor nodules. These effects are based on cytostasis, i.e., delayed proliferation of tumor cells and (or) their death (cytolysis). Many different techniques have been suggested and used for evaluating these events at the macro level: measurement and weighing of tumor nodules, counting the number of metastases in the lungs and liver, and so on. All these methods have two essential drawbacks. First, they take a long time (10-30 days or more). Second, the results obtained by them and the scatter of the data depend strongly on the health and general immunologic status of the mice, of their bacterial contamination, etc. Meanwhile, it has recently been shown that the most intensive processes of interaction between the tumor cell, on the one hand, and cells of the immune system, on the other hand, take place within the tumor itself, and also, possibly, in the regional lymph nodes [8]. The intratumor immune system is suppressed first and by the greatest degree [3], and its partial or total rehabilitation must lead to potentiation of the cytostatic and cytolytic action of effector cells of the immune system on the tumor cells.

The cytostatic effect is adequately evaluated in terms of reduction of the rate of proliferation. This estimation is relatively easy in vitro, but gives rise to problems in vivo (the need to inject large quantities of isotopes into the animals, subsequent measurement of their content in the tumor, etc.). The cytostatic effect is associated not only with reduction of incorporation of precursors into DNA, but also with inhibition of certain metabolic processes responsible for proliferation.

The aim of this investigation was to attempt to answer a number of questions. Can the cytostatic action be quickly and economically evaluated on a relatively small number of tumor cells, injected into the tumor itself? Can the rate of metabolism and elimination of ^{125}I-deoxyuridine (^{125}I-DU) from these cells be used as criterion of cytostatic action, bearing in mind that labeled products of iododeoxyuridine catabolism are reutilized to a lesser degree than degradation products of labeled thymidine [2, 5]?

EXPERIMENTAL METHOD

Mice of lines C57BL/6 and DBA/2, male and female and aged 3-4 months, were used. Tumors EL-4 and P-815 were used. Cells of leukemia EL-4 were transplanted intraperitoneally weekly into C57BL/6 mice, whereas cells of mastocytoma P-815 were injected into DBA/2 mice.

To obtain tumors, the cells were injected subcutaneously into the inguinal region of intact syngeneic animals, in a dose of $2 \cdot 10^6$ cells/mouse.

Research Institute of Human Morphology, Russian Academy of Medical Sciences, Laboratory of Cellular Immunopathology and Biotechnology, Moscow. (Presented by Academician of the Russian Academy of Medical Sciences N. K. Permyakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 7, pp. 68-70, July, 1992. Original article submitted December 28, 1991.

0007-4888/92/0007-1001$12.50

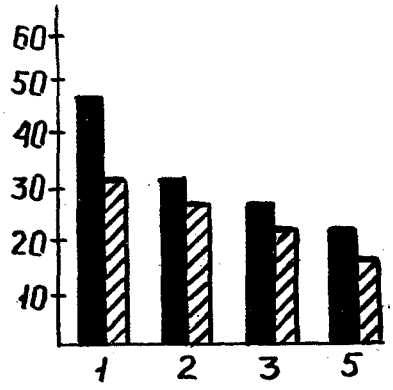

Fig. 1

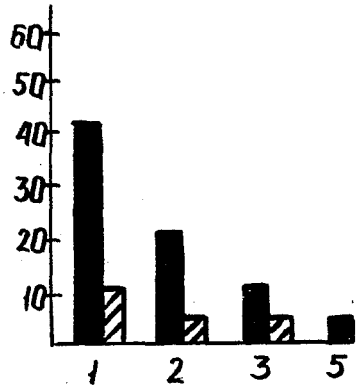

Fig. 2

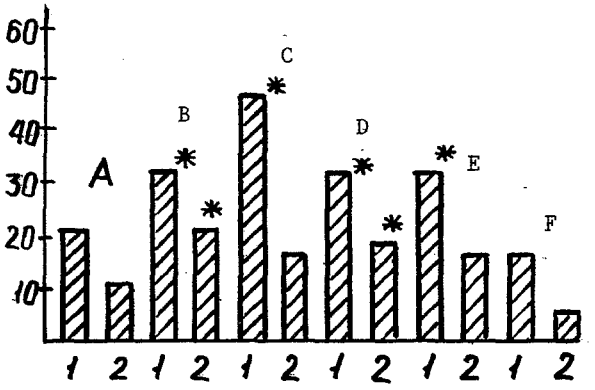

Fig. 3

Fig. 1. Dynamics of isotope elimination after injection of ^{51}Cr-labeled cells into tumor. Abscissa, days after injection of ^{51}Cr-labeled cells; ordinate, percentage of label remaining. Black columns – tumor EL-4 in C57BL/6 mice, oblique shading – tumor P-815 in DBA/2 mice.

Fig. 2. Dynamics of isotope elimination after injection of tumor cells labeled with ^{125}I-deoxyuridine. Abscissa, days after injection of ^{125}I-deoxyuridine labeled cells; ordinate, percentage of label remaining. Black columns – labeled cells injected into tumor, oblique shading – labeled cells injected subcutaneously into intact mice.

Fig. 3. Dynamics of isotope elimination under the influence of cytostatics. 1) 24 h after injection of labeled cells, 2) 48 h after injection of labeled cells. Ordinate, percentage of residual label in tumor. A) Control, B) intravenous injection of cyclophosphamide, C) intravenous injection of adriamycin (20 mg/kg), D) intravenous injection of adriamycin (4 mg/kg). E) Cells treated with actinomycin D, F) cells killed by freezing and thawing. Asterisk indicates significant differences between experiment and control ($p < 0.01$).

After 1 week, EL-4 and P-815 cells obtained from ascites fluid were labeled for 3 h at 37°C in medium RPMI containing glutamine and 10% fetal serum, and ^{125}I-DU (100 μCi/ml). In other experiments the tumor cells were labeled for 1 h with ^{51}Cr (100 μCi/ml). After incubation with the isotopes the cells were washed twice, resuspended in a large volume of medium, and allowed to stand for 1 h at 4°C (to ensure better removal of unincorporated isotope), and were then washed again twice. Some cells were treated with actinomycin D, and then washed off, and some were destroyed (by freezing and thawing three times). The cells were adjusted to a concentration of $2 \cdot 10^7$/ml and injected in a volume of 20 μl from a syringe into a tumor nodule in the opposite limb of the same tumor-bearing mice, and also into the inguinal fold of intact mice. As immunomodulators and cytostatics we used the following preparations: lipopolysaccharide (LPS) 7.5 μg/mouse ("Difco"); cyclophosphamide ("Sigma") 100 mg/kg; adriamycin ("WB") in doses of 20 and 4 mg/kg respectively; muramyl dipeptide (MDP, 15 μg/mouse); β-C_7H_{15}-MDP (200 or 50 μg/mouse); β-C_7H_{15}-MDP 200 μg/mouse; dexal-MDP (200 μg/mouse); polyacrylamide-MDP (200 μg/mouse), and polyacrylamide-MDP-phosphatidylethanolamine (200 μg/mouse). The last six preparations were synthesized in the Department of Organic and Analytical Chemistry, Simferopol' State University.

All preparations were injected intravenously simultaneously with injection of the labeled cells into the tumor (i.e., 7 days after subcutaneous inoculation of the tumor). NDP was injected together with LPS. The mice were killed 1, 2, 3, and 5 days after injection of the labeled cells. The tumor was removed together with surrounding tissues and radioactivity was determined on a γ-counter ("LKB").

The mass of the subcutaneous nodules was measured in separate control and experimental (LPS + MDP) groups 5 days after injection of the labeled cells, i.e., on the 12th day of growth.

EXPERIMENTAL RESULTS

A decrease in the intensity of isotopic labeling of cells labeled with ^{51}Cr in both models (P-815 and EL-4) can be seen in Fig. 1. A different picture was observed if the cells were labeled with ^{125}I-DU. In the course of 1 or 2 days the level of label in the tumor fell rapidly. If the labeled syngeneic cells were injected into intact animals, even more rapid elimination of the isotope was observed during the first 2 days (Fig 2). Elimination of the isotope from the tumor was then investigated after injection of a combination of LPS and MDP into the mice. During the first 2-3 days significant retention of label in the tumor was observed, after injection of the ^{125}I-DU-labeled cells into the tumor and subcutaneously.

After intravenous injection of the cytostatics, and also if the cells injected were treated beforehand with actinomycin D, delay of elimination of the label from the tumor also took place. If the cells were destroyed (by freezing and thawing three times) the isotope was eliminated more quickly from the tumor than in the control (Fig. 3).

We tested some new preparations of MDP derivatives. Since in the experiments described above the most significant results as regards delayed elimination of the label from the tumor were observed during the first 2 days after injection of the preparations, we used precisely that period to test the antitumor activity of MDP derivatives. Different MDP derivatives affected elimination of the label differently. For instance, the maximal effect was observed after intravenous injection of polyacrylamide-MDP-phosphatidylethanolamine and β-C_7H_{15}-MDP. This last preparation was used to compare two effects: delayed elimination of the isotope from the tumor and delay of tumor growth. The results showed that by the 11th day of development of the experimental EL-4 tumor its mass in the control was 4.6 ± 0.4 g, whereas in the experimental group after intravenous injection of β-C_7H_{15}-MDP it was 2.9 ± 0.3 g ($p < 0.01$).

Two directions of iododeoxyuridine metabolism in cells are known [6, 7]. First, phosphorylation and incorporation into DNA, and second, hydrolysis to iodouracil, followed by deiodination of the latter, for which nicotine-adenine dinucleotide phosphate is essential, and finally, incorporation of the free iodine into certain components of the cell, including into proteins. However, as Fig. 1 shows, proteins labeled with ^{51}Cr are eliminated comparatively slowly from the tumor. This suggests (Fig. 2) that reutilization of free ^{125}I into proteins during catabolism of iododeoxyuridine in the tumor cell is not significant. The process of catabolism of ^{125}I-DU and reutilization of iodouracil and of free iodine [1, 2, 7] is controlled by enzymes located in the soluble fraction of the cells [6].

It has been shown [5] that as early as 24 h after labeling of tumor cells growing in the peritoneal cavity, ^{125}I-DU and products of its catabolism and reutilization are absent from tumor cells, and that a further slow reduction of the label reflects actual loss of cells containing labeled DNA during the 5-15 days after their subcutaneous transplantation.

The results described above indicate that the rapid (in the course of 1-2 days) fall in the intensity of the label in a tumor into which tumor cells labeled with ^{125}I-DU in vitro also had been injected, reflects the catabolism of ^{125}I-DU and elimination of the products of catabolism from the tumor (or from the labeled tumor cells, injected subcutaneously). Inhibition of this process by immunomodulators and cytostatics in our experiments (data not given) in that case reflects inhibition of catabolism and elimination of its products, and also of products formed during reutilization.

It follows from Fig. 2 that catabolism and elimination of ^{125}I-DU take place more slowly if labeled tumor cells are injected into the tumor than if injected subcutaneously. As a first approximation the role of changes in the blood and lymph flow in this effect cannot be ruled out, but vascular changes usually did not develop so quickly [4], and naturally they should be different for the combination of LPS and MDP, on the one hand, and for cytostatics on the other hand.

We have compared the action of different MDP derivatives on catabolism and elimination of ^{125}I-DU from tumors. The most effective proved to be β-C_7H_{15}-MDP. Delay of ^{125}I-DU catabolism correlates in this case with significant delay of tumor growth, i.e., with the cytostatic action of the immunomodulator. According to Okumura and co-workers [5], the kinetics of ^{125}I-DU, incorporated into DNA, can serve as the criterion of cytolytic action on tumor cells.

The effect of immunomodulators on catabolism of ^{125}I-DU, when not incorporated into DNA, thus reflects their cytolytic effect. The use of ^{125}I-DU for comparative assessment of the cytostatic action of immunomodulators and cytostatics, as antitumor preparations, can sharply reduce the time and effort required in analysis of such preparations as the first step in their screening.

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