EFFECT OF ASCORBIC ACID ON INCORPORATION OF 5-FLUOROURACIL-6-^{3}H INTO ACID-SOLUBLE FRACTION AND RNA OF EHRLICH'S ASCITES CARCINOMA CELLS SENSITIVE AND WITH INDUCED RESISTANCE TO 5-FLUOROURACIL

M. P. Shlemkevich

UDC 615.277.3.015.21:615.356:577.164.2].015.44:616-006.6-008.949.4:615.277.3

KEY WORDS: 5-fluorouracil; ascorbic acid; RNA and DNA; Ehrlich's ascites carcinoma.

It has been shown [3] that the sodium salt of ascorbic acid increases fivefold the effect of 5-fluorouracil on neuroblastoma cells. Taking into account the results of that investigation, it was decided to study the effect of ascorbic acid on the rate of incorporation of 5-fluorouracil-6-^{3}H (5-FU-6-^{3}H) into the acid-soluble fraction and RNA of Ehrlich's ascites carcinoma cells sensitive and with induced resistance to that compound.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino mice weighing 20-25 g, into which an Ehrlich's ascites carcinoma, naturally sensitive to 5-fluorouracil (5-FU) was inoculated. The strain of the tumor resistant to 5-FU was obtained in mice into which this compound was injected intraperitoneally after inoculation of the tumor, in a dose of 15 mg/kg body weight daily. The strain resistant to 5-FU was obtained after 20 passages.

A 10% suspension of the tumor cells was prepared in Eagle's medium. 5-FU-6-^{3}H (specific activity 2.8 Ci/mmole, Czechoslovakia) was added to all samples in a dose of 1 μCi; the sodium salt of ascorbic acid was added to the experimental samples in a dose of 50 μg/ml medium. The intensity of incorporation of the preparation into the tumor cells was studied in these samples. In the second series of samples (prepared identically) the analysis was carried out 60 min after extraction of the preparation incorporated into the tumor cells in the course of the next 2 h. After the end of incubation the tumor cells were washed and homogenized in the cold in 0.5 N $HClO_4$. After extraction of the acid-soluble fraction DNA was separated from RNA [1]. The radioactivity of the samples was measured on a Mark II scintillation counter (Nuclear Chicago, USA).

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that incorporation of 5-FU-6-^{3}H into the acid-soluble fraction of the tumor strain sensitive to 5-FU was appreciably increased under the influence of ascorbic acid compared with the control. Incorporation of the compound into the acid-soluble fraction of the resistant strain of tumor was increased almost twofold by ascorbic acid compared with the control, and the level of incorporation not observed in cells of the sensitive strain until 60 min of incubation was already found after 30 min. Incorporation of the preparation into the acid-soluble fraction of tumor cells of the resistant strain decreased after incubation for 30 min, a characteristic feature of strains of tumors resistant to 5-FU [2]. After washing the cells to remove incubation medium (after 60 min) the compound was gradually and relatively steadily removed from the acid-soluble fraction of tumor cells of both sensitive and resistant strains.

As Fig. 2 shows, the intensity of incorporation of 5-FU-6-^{3}H into RNA was about an order of magnitude lower than into the acid-soluble fraction. Incorporation of the preparation into cells of the sensitive strain was approximately doubled by ascorbic acid compared with

Department of Biochemistry, L'vov Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. D. Ado.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 2, pp. 56-57, February, 1982. Original article submitted December 15, 1980.

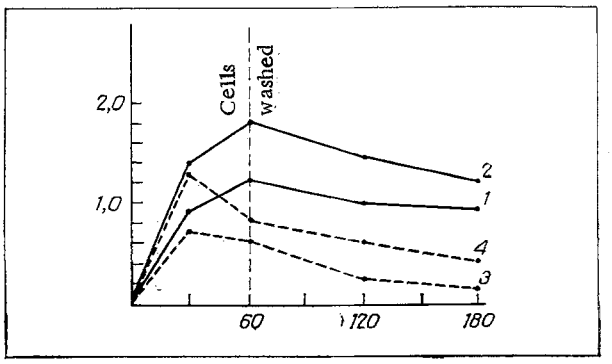

Fig. 1

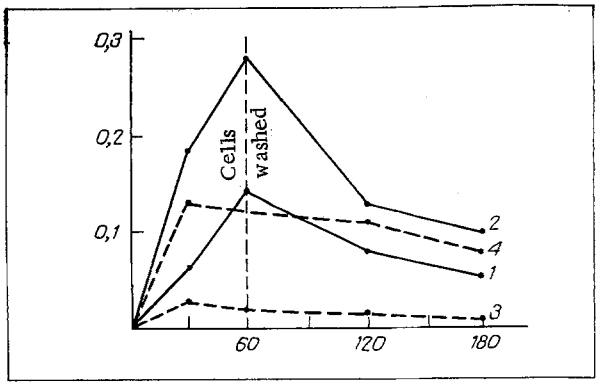

Fig. 2

Fig. 1. Incorporation of 5-FU-6-^{3}H into acid-soluble fraction of tumor cells of strains of Ehrlich's ascites carcinoma sensitive and with induced resistance to the compound. 1) Cells of sensitive strain of tumor in control, 2) cells of sensitive strain of tumor of incubation with ascorbic acid, 3) cells of resistant strain of tumor in control, 4) cells of resistant strain of tumor on incubation with ascorbic acid. Abscissa, incubation time (in min); ordinate, intensity of incorporation (in nmoles/mg DNA).

Fig. 2. Incorporation of 5-FU-6-^{3}H into RNA of tumor cells of strains of Ehrlich's ascites carcinoma sensitive and with induced resistance to the preparation. Ordinate, intensity of incorporation (in nmoles/mg DNA). Remainder of legend as to Fig. 1.

the control. Incorporation of 5-FU-6-^{3}H into RNA of the resistant strain was very low in the control, but under the influence of ascorbic acid, incorporation into RNA increased sharply and reached the characteristic level of the sensitive strain (in the absence of ascorbic acid). Elimination of the preparation from RNA of both strains of tumor cells took place quite uniformly, but more rapidly than from the acid-soluble fraction.

These experiments demonstrated the stimulating effect of ascorbic acid on incorporation of 5-FU into the acid-soluble fraction and into RNA of tumor cells of Ehrlich's ascites carcinoma sensitive and, what is particularly important, resistant to this compound. This effect is linked with the stimulating action of ascorbic acid on the cyclic AMP system [3], which evidently increases the permeability of cell membranes to 5-FU.

The results of these experiments also indicate the great practical importance of ascorbic acid when used in the chemotherapy of patients with 5-FU, for in ordinary therapeutic doses ascorbic acid behaves as a factor overcoming the resistance of tumor cells to this compound.

LITERATURE CITED

1. A. K. Belousova and G. K. Gerasimova, Vestn. Akad. Ned. Nauk SSSR, No. 5, 58 (1978).
2. K. Prasad, P. K. Sinha, M. Romanugam, et al., Proc. Natl. Acad. Sci. USA, 76, 829 (1979).
3. G. Schmidt and S. J. Thannhauser, J. Biol. Chem., 161, 83 (1945).