ACTION OF SOME CHEMICAL PREPARATIONS ON INTRACELLULAR DNA SYNTHESIS IN HUMAN STRAINS OF BREAST AND LARYNGEAL CANCER, CHORIONEPITHELIOMA, AND WILMS' TUMOR

E. S. Revazova

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The sensitivity of tumor cells to chemotherapy has now been the subject of much research. However, the study of the cell kinetics lying at the basis of mechanisms of the antitumor action of chemotherapeutic agents has been done mainly on animal tumors.

Investigation of the mechanisms of action of chemotherapeutic substances on the cells of solid tumors in man with the aid of labeled DNA precursors is difficult and has been carried out mainly *in vitro*, on tumor slices or in cell suspensions obtained from biopsy material.

A study of the action of 5-fluorouracil, methotrexate, cyclophosphamide, bleomycin, actinomycin D, and vincristine on DNA synthesis *in vitro* in suspensions of tumor cells with the aid of ^{3}H-thymidine and ^{3}H-deoxyuridine showed that incorporation of ^{3}H-deoxyuridine in tumors takes place more intensively than that of ^{3}H-thymidine, and activity of DNA synthesis correlates closely with the clinical effectiveness of the compounds [8]. In other investigations correlation was not found between incorporation of the label and sensitivity to the agents in patients [5].

The object of this investigation was to study the effect of chemotherapeutic agents on the intensity of DNA synthesis in some strains of human tumors obtained previously, and transplantable into thymectomized mice, by a radiometric method [2].

EXPERIMENTAL METHOD

Nude BALB/c mice, bred at the Oncologic Scientific Center and aged 1-1.5 months, were used. The following tumor strains were used: chorionepithelioma (CHE), carcinoma of the breast (CB), carcinoma of the larynx (CL), and Wilms' tumor (WT), which were transplanted subcutaneously in the form of cell suspension in Hanks' solution (ratio 1:3) in a volume of 0.5 ml. The compounds were injected when the tumors of all strains studied had attained a volume of 1000 mm^3. Altogether 12 compounds, injected once in maximal tolerated doses (Table 1), were studied. The compounds were injected intraperitoneally in 0.5 ml physiological saline. Only CCNU was dissolved in DMSO with Tween-80. The hormonal agent testosterone was injected subcutaneously in the form of a 50-mg tablet. The mice were given an injection of ^{3}H-thymidine, in a dose of 1 μCi/g body weight (specific activity 19.8 Ci/mmole) 21 h after injection of all the compounds except 5-fluorouracil and methotrexate. The action of 5-fluorouracil and methotrexate was studied with ^{3}H-deoxyuridine (specific activity 18.6 Ci/mmole), injected under the same conditions as thymidine. There were three control groups of animals: mice of group 1, the control for CCNU, received an injection of the solvent followed 21 h later by ^{3}H-thymidine; mice of group 2, the control for 5-fluorouracil and methotrexate, received an injection of ^{3}H-deoxyuridine; the mice of group 3, the control for the other chemotherapeutic agents, received an injection of ^{3}H-thymidine. The animals were killed 1 h after injection of the isotopes. A cell suspension was prepared from half of the tumors and treated by the method described previously [6]. Radioactivity was measured in a Mark II B Spectrometer and the number of counts per minute was determined. Student's and Fisher's methods were used for statistical analysis.

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TABLE 1. Intensity of Incorporation of Labeled DNA Precursors into Cells of Human Tumor Strains after Chemotherapy

Agents	Dose, mg/kg	Data of B spectrometer, cpm			
		CHE	CB	CL	WT
CCNU (2-chloroethyl-cyclohexyl-nitroso-urea	27	2875		2915	1849
NMU (nitrosomethylurea)	70	2378		2803	1799
5-Fluorouracil	93	0*	3719	—	—
Methotrexate	35	6508†	4078	3691	—
Vincristine	1,8	—	2982	—	1786
Vinblastine	1,9	—	—	—	1803
Bleomycin	55	—	—	2705	—†
Actinomycin D	0,2	782†	—	2681	695†
Prospidin	270	—	—	2695	—
Adriamycin	7,2	—	3213	—	—
Cyclophosphamide	100	—	3121	2793	1847
Testosterone	2000	—	629†	—	—
Control 1		2994	—	2973	1856
Control 2		3438	3946	3688	—
Control 3		2361	2805	2699	1781

*The compound was not studied.
†Statistically significant value by Student—Fisher test.

EXPERIMENTAL RESULTS

The preliminary autoradiographic observations showed that the cell kinetics of the strains of human tumors transplanted into nude mice differed only a little from that of the mouse and rat tumor strains described previously. Accordingly, the radiometric study was carried out 21 h after injection of the chemotherapeutic agents, for it is at that time that the greatest effect of chemotherapy on mouse and rat tumor strains is observed by this method. The high rate of growth of the tumors and the large number of proliferating cells in the strains used are favorable conditions for the study of dependence of activity of DNA synthesis on the effect of the compounds, for most chemotherapeutic agents act on proliferating cells. It will be clear from Table 1, which gives mean values for incorporation of the radioactive label in 4-7 mice, that more labeled thymidine was incorporated into cells of the CHE, CB, and CL strains, which have higher rates of growth and a greater proliferative pool than strain WT. In the present experiments the intensity of incorporation of the radioactive label was found to be clearly dependent on the effect of the chemotherapeutic agents on tumor growth in thymectomized mice [1]. This relationship was not observed only in the case of the action of cyclophosphamide on WT. The absence of effect of CCNU on the rate of growth of CHE also was reflected in incorporation of the radioactive label. Actinomycin D, which inhibits growth of the tumor node, reduced incorporation of the label by almost two thirds. Actinomycin D is known to block multiplying cells in the G_1 phase [3, 4]. By contrast with the direct dependence of the rate of growth of CHE on DNA synthesis, which we observed under the influence of actinomycin D, the opposite relationship between these values was found when methotrexate was used. Since methotrexate blocks multiplication of mouse tumor cells in the S phase [7, 9], it can be postulated that cells assimilate mainly in the S phase in this strain after administration of methotrexate. This in turn led to an almost threefold increase in incorporation of the label despite a decrease in size of the tumor.

The breast tumor transplanted into nude mice proved practically resistant to all the chemotherapeutic agents used but sensitive to testosterone. The inhibitory effect of testosterone, observed with respect to the radioactive label, can perhaps be explained by accumulation of cells in the G_1 phase in the tumor as the result of blocking of the transition of the cells from the G_1 phase to the S phase, as was observed when animal tumors were studied. Direct dependence of the size of the WT tumors on activity of incorporation

of label was observed when actinomycin D was used, just as during its action on the CHE strain, and it was due to blocking of the cells in the G_1 phase.

Unlike all the cases described above, during the action of cyclophosphamide on the WT strain, leading to a decrease in size of the tumors, DNA synthesis, measured 21 h after injection of the compound as the number of counts per minute, remained unchanged. It may be that at other times it would have been possible to detect some effect of cyclophosphamide on DNA synthesis in the WT strain. Just as in the previous investigation, in which the effect of the chemotherapeutic agent was assessed on the basis of their effect on the rate of tumor growth [1], strain CL proved insensitive to chemotherapy when the effect was assessed by the intensity of incorporation of the radioactive label.

Under the influence of actinomycin D on CHE and WT and also of testosterone on CB incorporation of the radioactive label was reduced as a result of blocking of the cells in the G_1 phase. The increase in the intensity of incorporation of the radioactive label observed under the influence of methotrexate was due to accumulation of cells in the S phase.

The blocking action of an antitumor agent on certain phases of the mitotic cycle of cells of human tumor strains transplanted into nude mice can thus be judged by studying changes in incorporation of radioactive label.

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