EFFECT OF SOME CHEMOTHERAPEUTIC AGENTS ON DNA SYNTHESIS AND DISTRIBUTION IN HUMAN STRAINS OF KIDNEY AND LUNG CANCER, MELANOMA, AND EWING'S SARCOMA TRANSPLANTED INTO NUDE MICE

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New methods of determining individual sensitivity of tumor cells to therapeutic agents are nowadays being used more and more often during investigations of patients. For this purpose, in clinical practice the method of continuous-flow cytophotometry is being used, by means of which it is possible to determine the redistribution of cells among phases of the mitotic cycle in tumors under the influence of chemotherapy [1, 2, 5].

Now that strains of human tumors transplantable into nude mice have been obtained [1, 4], it is possible to study the effect of chemotherapy on cell kinetics under more favorable conditions than with biopsy specimens of human tumors.

The object of the present investigation was to study the effect of chemotherapy on activity of DNA synthesis in cells and the distribution of cells by ploidy in human strains of lung and kidney cancer, melanoma, and Ewing's sarcoma.

MATERIALS AND METHODS

Strains of human tumors were transplanted subcutaneously into nude BALB/c mice. When the tumors had grown to 1000 m^3 the animals were given a single intraperitoneal injection of the agents in maximal tolerable doses, indicated in Table 1. From four to seven mice were used in a group. The chemotherapeutic agents were dissolved in physiological saline, but hexamethylmelanin (HMM) and 2-chloroethyl-cyclohexyl-nitrosourea (CCNU) were dissolved in dimethyl sulfoxide (DMSO) with Tween 80. The mice were given an intraperitoneal injection of ^{3}H-thymidine in a dose of 1 μCi/g body weight (specific activity 19.6 Ci/mmole) 21 h after injection of the chemotherapeutic agents, but after 5-fluorouracil and methotrexate they were given an injection of ^{3}H-deoxyuridine (specific activity 18.6 Ci/mmole). There were three control groups: animals of group 1, the control for CCNU and HMM, were given an injection of the solvent followed by ^{3}H-thymidine 21 h later; the animals of group 2, the control for 5-fluorouracil and methotrexate, were injected with ^{3}H-deoxyuridine; the animals of group 3, the control for the other agents, were given ^{3}H-thymidine. One hour after injection of the isotopes a suspension was prepared from half of the tumor and treated by the method of Nabholz et al. (1974). The intensity of incorporation of the radioactive label was measured by a Mark IIB spectrometer, and expressed in cpm. The other half of the tumor was homogenized, the cell suspension was fixed in ethanol and treated with pepsin in hydrochloric acid, and then stained with ethidium bromide. The ICP II pulse cytophotometer (PHYWE) was used for the investigation. The results of measurement of DNA were expressed in ploidy units, for which purpose the readings of the cytophotometer were calibrated against small human lymphocytes. Between 20,000 and 30,000 cells were studied in each tumor. From the DNA histograms thus obtained the relative number of cells (in %) containing different amounts of DNA was determined planimetrically. Student's t test was used for statistical analysis.

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TABLE 1. Effect of Chemotherapeutic Agents on Intensity of Incorporation of Radioactive Label into Cells of Human Tumor Strains

Agent	Dose, mg/kg	Data of B spectrometer, cpm			
		carcinoma of kidney	carcinoma of lung	melanoma	Ewing's sarcoma
CCNU	27	967*	1710	3005	—
Nitrosomethylurea (NMU)	70	— †	1662	2910	—
HMM	164	848	1675	—	3310
5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide	185	—	—	3016	—
Cyclophosphamide	100	—	1703	—	2816
Methotrexate	35	894	2491	—	—
5-Fluorouracil	93	901	—	—	—
Prospidin	270	815	1699	—	—
Bleomycin	55	763	—	—	—
Actinomycin D	0,2	856	—	2917	—
Vinblastine	1,9	752	—	2896	—
Vincristine	1,8	—	—	2903	—
Adriamycin	7,2	796	1689	—	2903
Sarcolysin	62	—	—	—	2947
Control I		804	2496	3119	3276
Control II		896	1714	—	—
Control III		759	1658	2859	2904

*Statistically significant value by Student's t test.

†Agent was not studied.

Legend. Control I) Solvent injected followed by ^{3}H-thymidine, control II) ^{3}H-deoxyuridine injected, control III) ^{3}H-thymidine injected.

EXPERIMENTAL

The data obtained by a study of the effect of the chemotherapeutic agents on the intensity of incorporation of the pulsed radioactive label in the tumors are shown in Table 1. Mean values obtained in experiments on 4-7 mice used in the control groups and to study each chemotherapeutic agent are given. The preliminary autoradiographic studies showed that the cell kinetics of these strains of human tumors, transplanted into nude mice, differed only a little from that of the mouse and rat tumor strains described previously. For that reason the cytophotometric and radiometric studies were carried out 2 h after administration of the chemotherapeutic agents, for it is at that time that these agents were found to have their greatest action on mouse and rat tumor strains. It was found that after 2 h only CCNU increased the intensity of incorporation of the radioactive label into the kidney cancer strain. All other agents had no action on incorporation of labeled isotopes by this strain. The increase in DNA synthesis in the kidney cancer strain under the influence of CCNU was not associated with any change in size of the tumor [3]. It was shown on transplantable mouse tumors that CCNU blocks cells in the S phase [6].

This causes an increase in labeling on account of accumulation of cells in the S phase, as is confirmed by the data of the DNA histogram (Fig. 1). Under the influence of CCNU the relative number of triploid cells, corresponding to the S phase, was increased fourfold.

Adriamycin was found to be active against Ewing's sarcoma. However, it did not change the intensity of incorporation of the radioactive label into Ewing's sarcoma cells. Accumulation of diploid cells and a fall in the number of triploid cells, corresponding to an increase in the number of cells in the G_1 phase and a decrease in their number in the S phase, can be clearly seen in Fig. 2. The increase in the number of cells in the G_1 phase can be explained by their blocking in this phase, which was found also in similar experiments with murine strains [5].

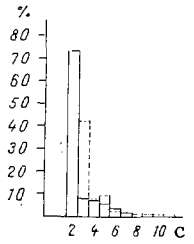

Fig. 1

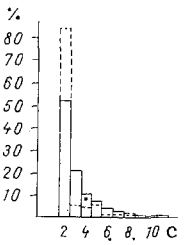

Fig. 2

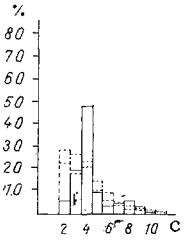

Fig. 3

Fig. 1. Distribution of cells of kidney cancer strain by ploidy after injection of CCNU. Here and in Figs. 2 and 3: horizontal axis — ploidy (in c units); vertical axis — number of cells (in %). Here and in Fig. 2: continuous line denotes control tumors; broken line tumors after administration of agent.

Fig. 2. Distribution of cells of Ewing's sarcoma strain by ploidy after administration of adriamycin.

Fig. 3. Distribution of cells of melanoma strain by ploidy after administration of actinomycin D and vinblastine. Continuous line — control tumors; broken line — tumors after administration of actinomycin D; dotted line — tumors after administration of vinblastine.

A study of the effect of chemotherapy on transplantable human melanoma showed redistribution of the cell populations by DNA content under the influence of actinomycin D and vinblastine (Fig. 3). Both agents increased the number of diploid and reduced the number of tetraploid cells. The increase in the number of diploid cells was attributable to their accumulation in the G_1 phase. Since melanoma strains were distinguished by their high heterogeneity, the changes in the redistribution of DNA under the influence of chemotherapy cannot be interpreted, more especially because the change in ploidy was not reflected at all in incorporation of label by the cells of that strain.

The strain of lung cancer was insensitive to chemotherapeutic agents with respect to all parameters studied: size of the node, distribution of cells by DNA content, and intensity of incorporation of the pulse radioactive label. Quantitative methods of study of cell kinetics (cytophotometry and radiometry) can be used to reveal the mechanisms of action of chemotherapeutic agents on human tumors transplantable into nude mice.

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