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EFFECT OF SOME CHEMOTHERAPEUTIC AGENTS ON DNA SYNTHESIS AND DISTRIBUTION IN TRANSPLANTABLE HUMAN GASTROINTESTINAL TUMORS

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To determine the mechanism of action of chemotherapeutic agents the study of cell kinetics by quantitative methods is widely used at the present time. With the appearance of continuous flow cytophotometry and B-spectrometry it is now possible to undertake mass cytological studies of tumors (A. S. Petrova). However, investigations of human tumor cell populations are conducted on biopsy material, which cannot give a complete answer to the question of the character and distribution of cells in a tumor nodule. An interesting model from this point of view is that of a human tumor transplanted into an athymic mouse. Although during the last ten years many investigators have studied transplantation of human tumors into athymic mice, so far only paper [6] on cell kinetics has been published. The reason may be difficulties in obtaining tumor strains with standard growth parameters.

The writers previously studied the duration of phases of the cell cycle, the size of the proliferative pool, and the character of ploidy in twelve original human tumor strains with stable growth characteristics. On the basis of the patterns of cell kinetics of human transplanted tumors thus revealed it was decided to study the effect of some widely used clinical chemotherapeutic agents on the cell kinetics.

The aim of the present investigation was to use cytophotometry and B-spectrometry to study the synthesis and distribution of DNA in cells of strains of human gastrointestinal tumors transplanted into athymic mice.

EXPERIMENTAL METHOD

Human strains of carcinoma of the large intestine (RTK-1 and RTK-2), carcinoma of the stomach (RZh), and carcinoma of the liver (RPech) were transplanted subcutaneously into

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TABLE 1. DNA Synthesis in Cells of Human Tumor Strains

Agent	Dose	Data of B-spectrometry, pulses/min			
		PTK-1	PTK-2	RZh	RPech
5-Fluorouracil	93	4107	3959	1897	3884
NMU	70	2531	2793	1224	3092
CCNU	27	2572	3014	1351	2453
Vinblastine	1,9	—*	—	—	2875
Vincristine	1,8	—	—	—	3106
Actinomycin D	0,2	—	—	—	1318
Adriamycin	7,2	—	—	—	2893
Methotrexate	3,5	—	—	—	3905
Control I		2497	2956	1268	2997
Control II		3549	3968	1929	4386
Control III		2698	3008	1348	2899

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

athymic BALB/c mice, reared by the authors, aged 1-1.5 months. The compounds were administered when the weight of the tumors of all strains studied was about 3 g. Three agents were studied and were injected once, in maximal tolerated doses (Table 1). In each group 4-7 athymic mice were used. 5-Fluorouracil and nitrosomethylurea (NMU) were injected intraperitoneally in physiological saline in a dose of 0.5 ml, whereas 2-chloroethyl-cyclohexene-nitrosourea (CCNU) was dissolved in dimethyl sulfoxide with Tween-80. An intraperitoneal injection of ^{3}H-thymidine (specific activity 19.8 Ci/mmole) in a dose of 1 mCi/g body weight was given 21 h after administration of CCNU and NMU, and ^{3}H-deoxyuridine (specific activity 18.6 Ci/mmole) was injected after 5-fluorouracil. Three control groups were used. The animals of the first group, the control for CCNU, were given an injection of the solvent followed, 21 h later, by ^{3}H-thymidine; the mice of group 2, the control for NMU, received ^{3}H-thymidine only; in group 3, the control for 5-fluorouracil and methotrexate, only ^{3}H-deoxyuridine was injected. The animals were killed 1 h after injection of the isotope. A suspension was prepared from half the tumor, treated by the method of Nabholz et al. [5]. Radioactivity was measured in cpm in a Mark II B-spectrometer.

To study the DNA distribution in the tumor cells the other half of the tumor nodule was homogenized to obtain a cell suspension. The cells were centrifuged at 1500 rpm for 5 min, resuspended in 0.9% NaCl solution, and fixed by the addition of 96% ethanol to a final concentration of 45%. To 2 ml of the cell suspension 2 ml of 0.5% pepsin in 0.2 N HCl was added and the mixture kept at 37°C for 15-20 min. Next, 0.1-0.5 ml of the cell suspension was added to a standard solution containing ethidium bromide (12.5 mg/ml) and the cells were stained at room temperature for 15 min. A JCF11 pulse cytophotometer (Phywe, Göttingen, West Germany) was used. The results of measurement of DNA in the tumor cells was expressed in ploidy units. For this purpose, the cytophotometer readings were calibrated against human small lymphocytes, the DNA content in which is about 2c. For each sample, 20,000-30,000 cells were measured in the cytophotometer with a flow rate of less than 500 cells/sec. The DNA content in the tumor cells was determined planimetrically from the DNA histogram thus obtained.

The statistical analysis was carried out by Student's test.

EXPERIMENTAL RESULTS

The experiments showed that only 5-fluorouracil, but not CCNU or NMU, inhibited growth of carcinoma of the human large intestine of strain RTK-1, transplanted into athymic mice. However, unlike the observations of Shy-Peck [7] and Fisman et al., who showed by autoradiography that the clinical effect of the agent correlates with its effect on DNA synthesis, this relationship was not discovered, contrary to expectations, during a B-spectrometric study of RTK-1 (Table 1). The results are in agreement with others showing that the quantity of label in solid tumors does not correlate with sensitivity to 5-fluorouracil [9]. According to other workers [8], who studied mouse tumors, 5-fluorouracil, which blocks cells in the S-phase,

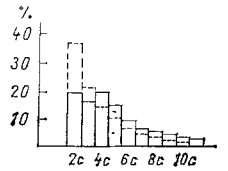

Fig. 1. DNA distribution in cells of strain RPech after administration of actinomycin D. Abscissa, ploidy (in c units); ordinate, number of cells (in percent). Continuous line — control tumors; broken line — tumors after administration of actinomycin D.

ought to promote the accumulation of cells in that phase and so increase incorporation of the radioactive label. According to other workers [4], 5-fluorouracil reduces the number of cells in the S-phase in transplanted tumors of the mouse large intestine. On cytospectrophotometry no action of 5-fluorouracil could be detected on the distribution of DNA in cells of carcinoma of the large intestine of strain RTK-1. In other experiments CCNU and MNU had no effect on DNA synthesis and distribution in this strain, by contrast with previous observations [4] showing that CCNU accumulates cells in the G_2 phase in mouse strain RTK.

None of the three chemotherapeutic agents studied inhibited growth of strains RTK-2 and RZh, and did not cause changes in DNA synthesis and distribution in these tumors.

The structure of populations of gastrointestinal tumor strains was thus unchanged by 5-fluorouracil, CCNU, and NMU.

DNA synthesis and distribution in strain RPech were found to be dependent on the action of actinomycin D, even though neither this substance nor any of the other seven agents tested caused any change in size of the tumors (Fig. 1). Other workers [2, 3] showed that actinomycin D, which simultaneously blocks proliferating cells in the G_1 phase and causes their death in the S phase, alters the ratio between the number of cells in different phases of the mitotic cycle. In human transplantable liver tumor RPech the same changes evidently took place, for the decrease in incorporation of radioactive label could be the result of accumulation of cells in the G_1 phase and a decrease in their number in the S phase. Because of the heteroploidy of cell populations of the RPech strain it was impossible to identify accumulation of cells in definite phases of the cycle. The only observation that could be made was that the number of diploid cells was increased under the influence of this agent.

The results of treatment of strains of gastrointestinal and liver tumors transplanted into athymic mice were thus rather contradictory. On the one hand, neither the synthesis nor the distribution of DNA was changed in tumors of strain RTK-1, which were reduced in size by 5-fluorouracil. On the other hand, DNA synthesis was inhibited and the DNA distribution changed in tumors of strain RPech under the influence of actinomycin D, although there was no change in their size during treatment. Nevertheless, changes in the DNA content and distribution detected by B-spectrometry and cytophotometry are evidence of a response of the tumor to the chemotherapeutic agent.

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