EFFECTS OF CHEMOTHERAPY AND RADIOTHERAPY ON GROWTH OF STRAINS OF HUMAN TUMORS OF ORGANS OF THE RESPIRATORY AND EXCRETORY SYSTEMS TRANSLATED INTO NUDE MICE

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The effects of antitumor agents on heterografts of human tumors in laboratory animals have been studied on various experimental models *in vivo*. The sensitivity of carcinomas of the lung and kidney, transplanted into immunodepressed mice, to methotrexate and hexamethylmelamine has been studied. The latter preparation proved to be more effective [1]. In other investigations in which tumor strains of human lung cancer transplanted into immunodepressed mice were used it was shown that carcinoma of the human lung is most sensitive to cyclophosphamide [2]. Reports have recently been published of the experimental treatment of tumors of the human lung transplanted serially into nude mice (tumor strain LX-1). Xenografts of lung cancer have been shown to be sensitive to 5-fluorouracil (5-FU) and cyclophosphamide [3]. According to data of other workers LX-1 is insensitive to cyclophosphamide and adriamycin [4]. Antitumor activity of 6-diazo-5-oxy-1-normycin (DON) and AT-125 (NSC 163501, an antimetabolite of glutamine) on xenografts of lung cancer LX-1 has been reported [5].

The object of this investigation was to study the effect of some preparations on strains of human tumors obtained by the writer and transplanted into nude mice: carcinoma of the lung (CL), carcinoma of the larynx (CLa), carcinoma of the kidney (CK), and Wilms' tumor (WT) [6]. The effect of radiotherapy on strain CLa also was studied.

EXPERIMENTAL METHOD

Experiments were carried out on nude BALB/c mice aged 1-1.5 months, bred in our own nursery. The tumors were transplanted by subcutaneous injection of a cell suspension. Strain CLa was transplanted after 14-15 days, strain CL after 33-35 days, strain WT after 24-26 days, and strain CK after 40-45 days.

Three schemes of injection of the preparations were used (Table 1). Animals of group 1 were given a single injection 48 h after inoculation of the tumor cells. Animals of group 2 also received one injection of the preparations: on the 8th day for strain CLa, on the 18th day for strain CL, on the 25th day for strain CK, and on the 10th day after inoculation of the tumor material for strain WT. The animals of group 3 received the preparations daily for 5 days: Animals with transplanted CLa tumors received the preparations from the 4th through the 8th day, those with CL from the 16th through the 20th day, with CK from the 25th through the 29th day, and those with WT from the 8th through the 12th day after transplantation of the cell suspension. All preparations were injected intraperitoneally in a volume of 0.5 ml. The maximal tolerated dose for nude mice was established beforehand. Each experimental group contained 6-15 mice, and altogether 910 animals were used.

Measurement of the tumors began on the day of injection of the compounds and ended at various times: 44 days after inoculation of the tumor for CL, 32 days after inoculation for CLa, 53 days for CK, and 36 days for WT. The effect of the preparations was determined from the kinetics of tumor growth and its percentage inhibition. The results were subjected to statistical analysis by the Student–Fisher method.

An attempt also was made in this investigation to treat the animals with transplanted CLa tumors by radiotherapy. Whole-body irradiation was given in one session on the 8th day after inoculation of the tumor, in a dose of 500 rads on an apparatus of the GuTr ^{137}Cs type.

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TABLE 1. Effect of Various Chemotherapeutic Agents on Tumor Strains

Preparation	No. of injections	Dose, mg/kg	Strain			
			CLa	CL	CK	WT
Actinomycin D	1	0,2	—	Not tested	—	+
	5	0,05	—	"	—	+
Bleomycin	1	55	—	"	—	Not tested
	5	22	—	"	—	"
Methotrexate	1	35	—	—	—	"
	5	1,9	—	—	—	"
Prospidine	1	270	—	—	—	"
	5	190	—	—	—	"
Hexamethylmelamine	1	164	Not tested	—	—	"
	5	84		—	—	"
Vinblastine	1	1,9		Not tested	—	—
	5	0,16			—	—
Vincristine	1	1,8			Not tested	—
	5	0,45				—
5-Fluorouracil	1	93			—	Not tested
	5	23			—	
Adriamycin	1	7,2		—	—	
Cyclophosphamide	1	100	—	—	Not tested	+
CCNV (2-chloroethyl-cyclohexylnitrosourea)	1	27	—	—	—	—
Nitrosomethylurea	1	70	—	—	Not tested	—

Legend. –) Negative result, +) positive result, statistically significant by Student–Fisher test.

EXPERIMENTAL RESULTS

Strain CK, when transplanted into nude mice, has a characteristic feature: Much of the tumor nodule is taken up by cavities filled with blood. The appearance of these cavities was observed from the beginning of tumor growth, Later the blood-filled cavities may occupy a considerable or the greater part of the tumor nodule, so that the quantity of actual tumor tissue varies considerably in different mice, and variations also are observed in the size of the module. This fact was reflected in the results of the study of the various antitumor agents. Statistically significant results could not be obtained with all the preparations used. Under the influence of most of them the CK tumors were always rather larger than in the control. However, this stimulating action was not statistically significant in each individual case.

Strain WT was much more sensitive to chemotherapy. Cyclophosphamide was the most effective agent under these experimental conditions (Fig. 1). When a single injection was given 48 h after inoculation of the cells, the appearance of the tumor node was delayed by 22 days. On the 27th day it measured not more than 55 mm^3. Meanwhile in the control tumors appeared during the first week and by the 8th day they had reached a volume of 55 mm^3, rising to 750 mm^3 on the 27th day. This delayed growth of the tumor after cyclophosphamide lasted until the end of the period of observation (36th day). The percentage inhibition of tumor growth at this time was 58.3. However, the rate of growth of the tumor in the animals of the experimental group was rather higher than in the control, and with time the differences between the values in the animals of the experimental and control groups must evidently disappear.

A single injection of cyclophosphamide into animals with tumors measuring 60 mm^3 (10th day after injection of the tumor cells) caused a sharp decrease in size of the nodule. The volume of the nodule 8 days after injection of the preparation was 35 mm^3, after which it began to increase, although marked retardation behind the control still remained until the 36th day, i.e., to the end of the period of observation. The rate of growth of tumors in animals receiving cyclophosphamide under these experimental conditions was not greater than in the control, and it is possible that this delay could continue in the future.

Another active preparation against WT was actinomycin D, which proved effective when administered by all three schemes. When injected 48 h after inoculation of the tumor suspension it delayed development of the tumor by 6 days (Fig. 2). However, the rate of growth of the developing nodule was so great that it soon became equal in size to the control (17th day). Later the tumor grew faster in the animals of the experimental group than in the control. In this case the small degree of stimulation of tumor growth in the second half of the period of observation was statistically significant.

Actinomycin D, if injected once into nude mice with a WT tumor measuring about 60 mm^3, gave a positive effect during the first 6 days after injection. The tumors in the experimental animals were smaller than those in the control for a further 10 days. However, the more rapid rate of growth of the nodules in the experimental mice than in the control led to abolition of the positive effect of the antibiotic.

Five daily injections of actinomycin D proved most effective: The decrease in size of the tumor nodule was most marked and prolonged, and it was observed as early as after the first injection of the preparation, to reach a maximum 6 days after the last injection. However, 11 days after injection of actinomycin D (the 23rd day after inoculation of the tumor) the nodule began to grow sharply and its rate of growth was much greater than in the control. Despite this fact,

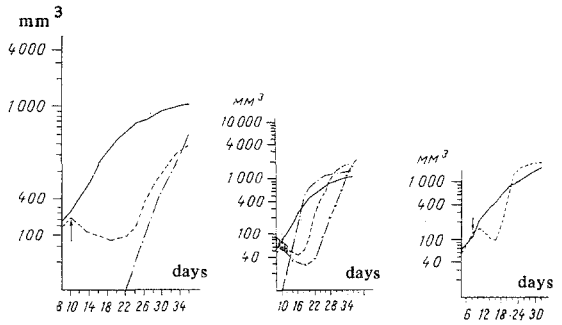

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Effect of cyclophosphamide on growth of strain WT. 1) Control, 2) 48 h after transplantation, 3) on 10th day after injection of tumor cells.

Fig. 2. Effect of actinomycin D on WT. 1) Control, 2) single injection 48 h after transplantation, 3) the same, 10 days after transplantation, 4) five injections on 8th-12th days after transplantation.

Fig. 3. Effect of a single irradiation (500 rads) on growth of strain CLa. 1) Control, 2) experiment.

the retardation of growth on the tumor nodule in the animals of the experimental group did not disappear until the 33rd day after transplantation.

A comparative study of the action of cyclophosphamide and actinomycin D on strain WT, as reflected in the percentage inhibition of growth showed that cyclophosphamide was the most effective substance. When injected into animals with tumors on the 10th day after transplantation both preparations produced equal inhibition of growth for 6 days. Later, however, inhibition of growth due to actinomycin D decreased and ceased altogether after 5 days. Meanwhile the positive action of cyclophosphamide continued for a further 2 weeks. Vinblastine, vincristine, CCNV, and nitrosomethylurea were ineffective against strain WT (Table 1).

Strains CLa and CL were most resistant to the action of all the chemotherapeutic agents studied: None of the preparations used, in whatever doses or schemes of administration tried, caused any statistically significant increase or decrease in tumor growth.

Irradiation of the animals with transplanted CLa tumors was carried out after the tumor had reached a size of 140 mm^3. During the first 2 days after irradiation the tumor continued to grow (160 mm^3). During the next 6 days its size decreased to 100 m^3 (Fig. 3). From the 8th day after irradiation, however, the nodule began to grow rapidly, and after 13 days it was as big as the control tumor, and later it exceeded it in size a little.

Consequently, the results indicate that most of the chemotherapeutic agents tested were ineffective for the treatment of human CLa, CL, and CK when transplanted into nude mice. Meanwhile there is evidence to show that lung tumor strain LX-1 was sensitive to other preparations, which were not studied in these experiments: DON, AT-125, and melphalan [5]. Unlike Giavanella et al. [3], we did not observe a positive effect of cyclophosphamide on strain CL, in agreement with other workers [4].

Radiotherapy had a positive but temporary action on CLa in a dose of 500 rads, the maximal dose tolerated by mice. These results correlate with the observed radiosensitivity of human laryngeal tumors.

The cause of the almost total resistance of CK to chemotherapy may perhaps lie in the structural characteristics of the tumor. The presence of blood-filled cavities in the nodule and the considerable scatter of the quantity of tumor material in the neoplasms do not allow any definite effect of the various preparations to be detected. These data do not agree with the reports published by other workers [1] on the efficacy of hexamethylmelamine on tumors of the human kidney and lung in immunodepressed mice.

It is a well known fact that WT in man is sensitive to many chemotherapeutic agents, and this was confirmed in the present investigations. Administration of actinomycin D and, in particular, of cyclophosphamide was followed by inhibition of tumor growth in nude mice. However, complete regression was not observed in any scheme of treatment.

The contradictory nature of the results obtained by ourselves and other workers on the action of different therapeutic agents can evidently be explained by two principal causes: first, the individual sensitivity of the human tumors, and second, the great diversity of the experimental conditions. It is difficult to compare our own data because of differences in the sources from which the tumors were obtained for transplantation, differences in the doses of the agents used, in the conditions of keeping of the nude mice, the methods of grafting, and so on.

The results confirmed the individual sensitivity of tumors transplanted into nude mice to chemotherapy and radiotherapy, and this corresponds to their sensitivity in man to treatment under clinical conditions [3]. In further investigations it will be necessary to have series of tumors of the same localization transplanted into nude mice in order to discover the most general rules.

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RESISTANCE-DEPRESSING AND METASTATIC ACTIVITY OF TRANSFORMED SYRIAN HAMSTER CELLS

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Evidence in support of the view that a tumor cell population is heterogeneous with regard to its metastasizing activity was obtained some time ago [5, 6]. This hypothesis was recently proved [4, 7]. According to the authors cited, the formation of visible metastases is the result of *in vivo* selection of a cell subpopulation which possesses certain properties essential for passage through all the stages of selection. It is evident that in the early stages of the metastatic process selection of cell variants will be based on a number of different features. It is still not clear what biological properties determine the ability of tumor cells to overcome each barrier on the road to metastasis formation. There are several such barriers in the path of spread of tumor cells from the primary focus into the blood vessels and lymphatics, until they penetrate into the tissues of different organs. The formation of metastases actually in the organs and tissues is evidently associated with the surmounting of other barriers. In our view [1-3], one of the essential properties of tumor cells for metastasis formation is their ability to depress the natural resistance (NR) of the host to the tumor. In previous experiments on Syrian hamsters the present writers showed that inactivated cells of various tumor strains (unlike normal cells) have the ability to depress the NR of the host to the tumor, and a system for determining the resistance-depressing activity of tumor cells *in vivo* was worked out [1]. It was postulated that tumor cells contain a thermostable factor capable of depressing the NR of animals to tumors and that this factor is important for the development of monoclonal tumors, for metastasization of tumors, and also for tumor growth after transplantation of single tumor cells [1-3].

Ability to depress the NR of animals to tumors was discovered during the investigation of a number of hamster tumor strains of varied origin, but it was not found when normal Syrian hamster embryonic cells were studied in the second half of embryogenesis, or in cells of a strain of embryonic hamster cells transformed spontaneously *in vitro* (strain HETR). Meanwhile the cells of this strain, if transplanted subcutaneously into hamsters in large doses, can form metastases in the lungs.

In the investigation described below the resistance-depressing activity (RDA) of the parental HETR strain and of a number of its sublines obtained from distant lung metastases, and also their metastasizing activity were studied.

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