INVESTIGATION OF THE ACTION OF A NUMBER OF ANTITUMOR PREPARATIONS ON CULTURES OF HUMAN TUMORS

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Translated from Byulleten' eksperimental'noi biologii i meditsiny Vol. 49, No. 1, pp. 95-98, January, 1960

Original article submitted January 28, 1959

In the solution of problems concerned with the choice and the determination of the spectrum of antitumor preparations, transplanted animal tumors are used. Human and animal tumors, even of the same histological structure, show differing sensitivity to these preparations, however, and investigations on animal tumors do not furnish sufficient evidence on which to form conclusions on the efficacy of a drug in particular tumors in man. It is therefore necessary to seek new methods of determining, directly on human tumors, the spectrum of action of each new preparation which has been found active against animal tumors.

The use of explantation of tissues for this purpose shows great promise. The applicability of the method of cultivation of human tumors is based on the fact that, from many reports, the mechanism of the antitumor action of the majority of chemotherapeutic preparations is by their direct action on tumor cells [1, 6]. It follows that the antitumor activity of a given preparation against a given form of tumor can also be detected in vitro. Furthermore, the technique of cultivation of human tumors is now so well developed that strains of the principal human tumors are at present available for research workers, and methods have been devised for the production of single-layer cultures from biopsy material. Preparations may be tested on human cultures for periods of 30-40 hours.

The recent literature contains a number of recommendations by the leading authorities on tissue culture concerning the use of this method in the chemotherapy of tumors [4, 7, 8]. Nevertheless, in the first attempts to use the tissue culture method to detect antitumor activity in these preparations, contradictory results were obtained. Certain workers, for instance, observed differences in the sensitivity of cultures of tumor and normal tissue to the same preparation [5], whereas in other, similar investigations, no selective activity of the preparations could be found (see the survey by Hirschberg [10]). One reason for the contradictory nature of these results is, evidently, the fact that the preparations used in the investigations giving negative results possessed high toxicity, which could have reduced the sensitivity of the tumor and normal tissue to the antitumor action of the preparations.

It is also possible that other causes may be implicated in determining the results of these investigations.

Meanwhile the problem of the detection of the specific antitumor action of preparations in tissue culture conditions remains unsolved. The aim of the present investigation was to study this problem.

METHODS

A special feature of our experiments is that, besides studying preparations already known, we also tested new ones, created in accordance with the principle of complex alkylating metabolites, enunciated by L. F. Larionov [2], namely asalin, phenaphan, and phenameth. In animal experiments these drugs showed high antitumor activity, a varying degree of specificity of their action on tumors [3] and, at the same time, comparatively low toxicity.

The chemical names of these preparations are as follows: asalin is N-acetylsarcolysinvaline (ethyl ester); phenaphan is p-di(2-chloroethyl) aminophenacetylphenylalanine (ethyl ester); phenameth is p-di(2-chloroethyl) aminophenacetylmethionine (ethyl ester).

We also investigated the action on tissue cultures of acetylsarcolysin and chlorophenacyl [p-di(2-chloroethyl) aminophenylacetic acid], which enters into the composition of these three preparations, and also of three previously known preparations – embichin, DL--sarcolysin, D-sarcolysin, and thio-TEPA.

We had available cultures of a strain of carcinoma of the uterine cervix (HeLa), obtained from Gey [9], cultures of normal epithelium from a human embryo (from A. D. Timofeevskii's laboratory 1958), and also cultures of the normal endothelium of the heart of a Cinomolgus monkey, obtained in the USSR in D. E. Solk's laboratory in 1957. First of all we attempted to discover any differences in the sensitivity of tumor and normal

tissue to equal doses of the same preparation under explantation conditions, i.e., if there was any selectivity in the action of the preparation on the cultures of tumor cells.

The strains used were cultivated by the usual method on synthetic medium 199, with the addition of human serum (20%) for carcinoma of the cervix uteri and for normal human epithelium, and also ox serum (20%) for the endothelium. Cultivation was carried out in Carrel flasks, 4-5 cm in diameter. After growth for 5 days, the prospective preparations were added in different doses to the fresh nutrient medium of the experimental cultures. The period of action was 24-48 hours, after which the cultures were photographed; next, a proportion of the flasks was treated with trypsin solution (DIFCO), the detached cells were centrifuged at 1000 rpm for 3 minutes, fresh nutrient medium was added to the precipitate, and the experimental cultures were again allowed to grow. The results of the action of the preparations were assessed by the ability of the culture to grow in the second generation. The maximum dose at which the cells grew in the form of colonies was defined as the "maximum dose of positive passage". If the cells were found to be incapable of further growth in new flasks, the minimum dose of the preparation causing these changes was designated the "minimum lethal dose". Naturally, the more important value in defining the antitumor activity of the preparations was the maximum dose of positive passage, and this value was compared in different series of experiments.

RESULTS

The results obtained from our experiments are shown in Table 1. It can be seen that the cultures of normal epithelium and endothelium possessed different sensitivity to the test preparations. From a comparison of the action of the same preparations on carcinoma of the uterine cervix and on epithelium, for instance, the highest selective antitumor action was shown by phenaphan and acetylsarcolysin; in response to the action of these preparations, the tumor culture retained its power of growth in the second generation only with doses of 125 and 100 γ/ml, whereas the culture of epithelium was capable of passage after the action of doses as high as 500 γ/ml of phenaphan and 400 γ/ml of acetylsarcolysin. Comparative evaluation of the action of these two preparations on carcinoma of the uterine cervix and on endothelium showed that asalin had the highest selectivity of action; after the action of this preparation a second generation of tumor cells grew only with a dose of 50 γ/ml, but cultures of endothelium kept the power of passage even with a dose of 200 γ/ml.

It follows from Table 1 that 6 of the 9 preparations studied, namely all the alkylating metabolites except DL-sarcolysin, had a stronger action on the culture of tumor cells than on the cultures of normal cells, for the maximum dose of positive passage was half as great for the tumor cells as for normal cells.

TABLE 1 Comparative Action of Chemical Preparations of the Chloroethylamine Group on Cultures of Malignant and Normal Cells

Preparation	Maximum dose of positive passage		
	carcinoma of the cervix (HeLa)	human epithelium	endothelium of the monkey's heart
Embichin	25	12.5	12.5
DL-Sarcolysin	25	25	25
D-Sarcolysin	100	200	200
Chlorphenacyl	50	50	200
Phenaphan	125	500	250
Phenameth	100	200	200
Asalin	50	100	200
Acetylsarcolysin	100	400	200
Thio-TEPA	50	100	50

TABLE 2 Comparative Action of Chemical Preparations of the Chloroethylamine Group on Different Strains of Human Malignant Tumors in Tissue Culture

Preparation	Maximum dose of positive passage (in γ/ml)		
	carcinoma of the uterine cervix (HeLa)	carcinoma of the larynx (HEr = 2)	angiosarcoma (AS)
Embichin	25	12.5	12.5
DL-Sarcolysin	25	12.5	25
D-Sarcolysin	100	50	50
Phenacyl	50	50	50
Phenaphan	125	125	1000
Phenameth	100	100	400
Asalin	50	50	200
Thio-TEPA	50	25	50

It is also apparent from Table 1 that DL-sarcolysin had no selective action on the tumor cultures, and that embichin had a stronger action on the epithelium and endothelium than on the tumor cells. So far as chlorophenacyl and Thio-TEPA are concerned, the selectivity of action of Thio-TEPA was shown by comparison of its action on tumor cultures and cultures of normal epithelium, and the antitumor action of chlorophenacyl was shown by comparison of its action on the same tumor cells and the culture of endothelium.

The new antitumor preparations which we studied, belonging to the group of "complex alkylating metabolites", thus possess greater selectivity of action than the antitumor preparations previously known.

The difference in the sensitivity of the tumor and normal tissues to these antitumor preparations during explantation enabled us to determine the spectrum of action of these preparations on human tumors in these

conditions. We had available 3 strains of human malignant tumors — carcinoma of the uterine cervix (HeLa), carcinoma of the larynx (HEr = 2) and angiosarcoma (AS), and these were used in the investigation. The results obtained are shown in Table 2.

From a comparison of the maximum doses of positive passage it can be seen that 2 of these strains — — carcinoma of the uterine cervix and carcinoma of the larynx — were more sensitive to 3 of the 8 preparations tested, namely to the "complex alkylating compounds" phanaphan, asalin and phenameth, whereas angiosarcoma continued to grow in the second generation even after the action of a large dose. It may be postulated from these findings that these particular preparations showed greater specificity of action on certain variants of squamous-cell carcinoma than on sarcoma. With regard to the preparations used, the method of tissue culture of human tumors thus gave a provisional answer to the question of which human tumors might be expected to show therapeutic effects from the use of these preparations.

Our experiments also revealed a difference in the action of the preparations on the different epithelial tumors. Carcinoma of the larynx, for instance, was more sensitive to D-sarcolysin and thio-TEPA than was carcinoma of the uterine cervix.

It follows from these results that we succeeded in demonstrating an appreciable difference in the maximum doses of positive passage of certain of the antitumor preparations when used on tissue cultures of human tumors. The suggestion, made above, that it may be possible to use the tissue culture method to detect not only antitumor activity but also the spectrum of action of the preparations examined, was thus confirmed. Comparison of our results on cultures in vitro with the results of clinical trials of these particular preparations will indicate to what extent this method may be used for practical purposes.

SUMMARY

Experiments were conducted on cultures of human tumor cells — of cancer of the uterine cervix (HeLa), cancer of the larynx HEr-2, and angiosarcoma (AS), as well as on those of cardiac endothelium of the "Cinomolgus" monkey. The author studied the action of the new Soviet antitumor preparations asalin, phenaphan, phenameth, acetylsarcolysin, and phenacyl, as well as of the existing preparations — embichin, dl-sarcolysin, d-sarcolysin and thio-TEPA. Investigations revealed that differences in the sensitivity of the tumor and normal tissue to the same preparations are also present in tissue culture. Thus, the effect of the complex alkylating metabolites (excepting dl-sarcolysin) on tumour cell cultures was 2—4 times greater than that exerted on cultures of normal cells. The varying effect of the preparations under study on different tumors could be also ascertained in tissue cultures. Thus, carcinoma of the uterine cervix and of the larynx appeared to be more sensitive to asalin, phenaphan, and phenamethane than did angiosarcoma, while the carcinoma, of the uterine cervix proved to be more resistant than carcinoma of the larynx to d-sarcolysin and Thio-TEPA.

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