EXPERIMENTAL DETERMINATION OF INDIVIDUAL SENSITIVITY OF TUMORS TO ALKYLATING COMPOUNDS WITH ANTITUMOR ACTION

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Experiments on rats revealed a direct relationship between inhibition of tumor growth in vivo by the alkylating agents thiophosphamide, benzo-TEP, cyclophosphamide, and sarcolysin, and the decrease in content of SH-groups produced by the action of these compounds on tumors in vitro. The results were used as a basis for the detection of individual sensitivity of tumors to alkylating compounds with antitumor action.

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Numerous clinical observations have shown that tumors in the same situation, and even with the same histological structure, frequently differ in their sensitivity to therapeutic agents. This presents a most important practical problem, namely that of determining the sensitivity of a tumor to antitumor compounds. So far, however, the mechanism of tumor cell metabolism, responsible for the very wide differences in the response of tumors to antitumor agents, have not yet been explained. Methods so far suggested for determining tumor sensitivity to compounds have not been applied in clinical practice [5-10].

The writer has previously shown [1, 2] that under the influence of alkylating antitumor compounds the content of tissue sulfhydryl groups falls sharply in tumors sensitive to these compounds 15-30 min after parenteral administration of the cytostatic agent, and the degree of inactivation of the tumor is directly dependent on its sensitivity to the compound used. Analysis of the results obtained suggested, first, that the first reaction of alkylating compounds with tumor SH-groups is associated with a direct reaction of alkylation of the tumor SH-groups by the compound; second, that the degree of this reaction depends on sensitivity of the tumors to the alkylating compounds. Remembering that there is no difference between accumulation of alkylating compounds in sensitive and drug-resistant tumors [3, 11], it therefore seems likely that the SH-groups of tumors differ in their ability to react with antitumor compounds, and this may perhaps to some extent determine the sensitivity of the tumor to the compound, and can perhaps be detected by experiments in vitro.

The present investigation was carried out to study the antitumor activity of a compound in vivo on a particular strain of tumor and the degree of inactivation of the SH-groups of that tumor in experiments in vitro under the influence of the same compound.

EXPERIMENTAL METHOD

Experiments were carried out on 200 male albino rats weighing 130-150 g with Guerin's carcinoma and with a substrain of this tumor with induced drug resistance [3], resistant sarcoma 45, and sarcoma M1. The tumors, differing in their degree of sensitivity to the various alkylating antitumor compounds, were injected subcutaneously into the animals. On the 8th-10th day after inoculation, one of the following compounds was injected into the animals of different groups: thiophosphamide (2.5 mg/kg), benzo-TEP (15 mg/kg), cyclophosphamide (10 mg/kg, intramuscularly, daily for 10 days), and sarcolysin, (5 mg/kg on alternate

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TABLE 1. Content of SH-Groups in Tumor Tissues after Treatment with Compounds in vitro (in percent of initial level) and Inhibition of Tumor Growth in vitro (in percent)

Strain of tumor	Thiophosphamide		Benzo-TEP		Sarcolysin		Cyclophosphamide	
	Content of SH-groups in vitro	inhibition of growth	content of SH-groups in vitro	inhibition of growth	content of SH-groups in vitro	inhibition of growth	content of SH-groups in vitro	inhibition of growth
Guerin's carcinoma	55,0±3,3	92	50,7±3,7	99,3	60,0±2,6	85	68,0±5,1	76
The same (resistant)	84,0±4,8	34	85,0±3,3	36	85,0±2,2	22	92,0±5,6	10
Sarcoma 45 (resistant)	87,0±4,6	34	79,0±3,6	56	84,0±2,2	45	82,0±3,1	38
Sarcoma MI	60,0±3,6	79	50,3±4,1	92	56,0±5,0	82	73,0±1,7	56

days in 5 doses). The rats were sacrificed after the end of treatment, the tumors removed and weighed, and the percentage of inhibition of their growth was determined by the usual method.

The degree of inactivation of SH-groups in the tumor tissue under the influence of these compounds was determined in control animals of each series in experiments in vitro. Weighed samples of tumor tissue, free from necrotic areas, were minced in Potter's homogenizers with cold physiological saline. The total content of SH-groups in the supernatant of the homogenate containing SH-groups of water-soluble proteins and of low-molecular weight compounds was determined by amperometric and mercurimetric titration. The compounds were then added to test tubes containing 1 ml of homogenate and incubated at 37° for 1 h. After incubation the level of total SH-groups was determined each sample and the degree of their fixation by the compound was estimated by experiments in vitro relative to the level of SH-groups in the control sample before incubation. To obtain comparable results, in the experiments in vitro it was necessary to test the action of the compounds in doses producing the same biological effect as in the experiments in vivo. For this reason, the concentrations of the compound in the test tubes were made equal to those produced in the rats' body after administration of a dose of compound equal to LD_{50}, assuming its uniform distribution throughout all organs and tissues.

EXPERIMENTAL RESULTS

The results obtained are given in Table 1. If the percentage of inhibition of tumor growth is compared with the degree of lowering of the content of SH-groups in the tissues by the same compounds, a definite correlation can be seen between these indices. For instance, Guerin's carcinoma was most sensitive to the action of this group of compounds. Benzo-TEP inhibited its growth by 99%, and in the experiments in vitro the content of SH-groups in the tissue of Guerin's carcinoma was lowered by this compound by 49.3%. In experiments carried out in the same manner growth of Guerin's carcinoma with induced drug-resistance was inhibited by benzo-TEP by 36%, the level of tissue SH-groups of the tumor under these circumstances being reduced in vitro by this compound by only 15%. Thiophosphamide inhibited growth of Guerin's carcinoma by 92% and the content of tissue SH-groups in this experiment in vitro was reduced by 45%. The content of SH-groups in tissues of the drug-resistant type of Guerin's carcinoma was reduced by thiophosphamide by 16%, while growth of this tumor was inhibited by the compound by only 34%. As Table 1 shows a similar relationship can be observed by analysis of data obtained with all four strains of tumor and under the influence of all compounds.

In tissues of the same tumor, the decrease in level of SH-groups was more marked under the influence of that compound which inhibited growth of that tumor more strongly after repeated administration. For instance, benzo-TEP inhibited growth of sarcoma M1 by 92%, while cyclophosphamide did so by 56%; the content of SH-groups in the tissues of this tumor was lowered by 49.7 and 27%, respectively. Similar comparisons can be made from the data given in Table 1.

It can concluded from the results obtained that there is a clear relationship between the degree of inhibition of tumor growth in animals after repeated administration of a compound and the decrease in content of tissue SH-groups in that tumor under the influence of the same compound in vitro.

On the basis of the results obtained in vitro, it is considered that the method described above can be recommended for determination of individual sensitivity of human tumors to alkylating compounds with antitumor action.

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