Reaction of Pulmonary Tumor Transplants to Irradiation and Platidiam in the Subcapsular Test

O. I. Skotnikova, N. S. Sergeeva, E. V. L'vova, V. I. Chissov, and I. I. Pelevina

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Effects of γ-irradiation in a dose of 6 Gy and platidiam in a total dose of 16 mg/kg on the growth rate of transplants of 20 human pulmonary carcinomas under the renal capsules of immunodepressed mice were studied. Ten out of 11 tumors resistant to radiation were found to be resistant to platidiam as well. On the other hand, only 10 out of 15 carcinomas resistant to platidiam were resistant to radiation.

Key Words: *irradiation; chemical agents; pulmonary carcinomas; subcapsular test*

By now several cellular systems responsible for the chemical resistance and radioresistance of tumor cells have been described, among which are the systems of antiperoxide defense [2], of DNA repair enzymes [10,12], and some others [3,11].

Initial cross-resistance to platinum drugs and radiation has been demonstrated for some transplant cell lines [13]. In addition, some scientists have shown parallel development of resistance to platidiam by tumor cells upon their irradiation [8]. These data indicate the possibility of activating common mechanisms of resistance to radiation and some cytostatics in cells of certain types. It is not clear, however, whether coupled chemo- and radioresistance is characteristic of human primary carcinomas.

Several experimental approaches to the detection of primary chemo- and radioresistance of human carcinomas are known, e.g., the clonogenic, radiometric, and microtetrazolium tests [1]. The subcapsular test (ST) is considered to be one of the best models for the detection of human tumor chemical resistance [5,7]. The method consists in assessing the growth parameters of a human carcinoma transplant under the renal capsule of an animal immunodepressed by γ-radiation and administered chemotherapy. We adapted this method for the detection of primary radioresistance of human carcinomas [4].

In this study we used the ST to investigate cross chemical and radioresistance of human pulmonary carcinomas.

MATERIALS AND METHODS

Twenty pulmonary carcinomas (13 squamous-cell carcinomas, 2 small-cell carcinomas, and 5 adenocarcinomas) obtained after surgical intervention were examined. Under aseptic conditions tumor material was placed in medium 199 with 1% penicillin and streptomycin and left for 14-18 h at 4°C.

Male CBA mice weighing 19-21 g were used as human tumor recipients. A day before transplantation the animals were exposed to a single total γ-irradiation in a dose of 4 Gy to suppress the graft rejection reaction. Tumor tissue was cut into 2-3 mm^2 fragments, some of which were exposed to a single γ-irradiation in a dose of 6 Gy. The irradiated and intact fragments of the tumor were then transplanted under the renal capsule of animals (intact fragments to controls and experi-

P. A. Hertzen Moscow Research Oncological Institute, Ministry of Health of Russia; Institute of Chemical Physics, Russian Academy of Sciences, Moscow

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mental group 1 animals, irradiated fragments to experimental group 2 animals). Each group consisted of 7-9 animals. Control animals were daily injected 0.2 ml normal saline intraperitoneally, starting from day 2 after transplantation (a total of 4 injections). Group 1 animals were injected platidiam after the same protocol in a single dose of 4 mg/kg.

The effects of irradiation and platidiam on each tumor were assessed by comparing the mean relative increment of the graft size (in 6 days) in controls with the relative change in graft size in experimental groups of animals and expressed in percent of tumor growth inhibition or regression [5]. The tumor was considered to be sensitive to the agent in question (platidiam or radiation) if this agent caused transplant regression, and resistant if it just inhibited or stimulated transplant growth .

The quality of tumor tissue taken for transplantation and the cellular composition of 6-day transplants were assessed by the cytological method on smears prepared from these tissues and stained after Pappenheim [6].

The reliability of differences in the data was estimated by calculating the standard deviation for each value.

RESULTS

The mean relative increment in the size of pulmonary carcinoma transplants under the renal capsule of mice differed within a wide range: from 0.18 to 1.2, this corresponding to the mean increase in the size of the transplant in 6 days by 18 to 120% *vs.* its initial size, the mean value being 0.57. The reaction of tumor transplants to preirradiation in a dose of 6 Gy was individual as well - from 40% regression to 205% stimulation of tumor growth (Table 1). For example, radiation treatment led to regression of 9 out of 20 tumor transplants and inhibited the growth of 3 out of 20 grafts; in the remaining cases irradiation stimulated tumor growth under the renal capsule (Table 1). The reaction of pulmonary carcinomas to platidiam in ST was also individual: from 48% regression to 259% stimulation of tumor growth (Table 1). Injection of platidiam to animals led to regression of 5 out of 20 tumors, to growth inhibition to various degrees in 12 tumors, and to growth stimulation in 3 tumors.

The ratio of different variants of pulmonary carcinoma sensitivity/resistance to radiation and platidiam (considering as effective an exposure causing transplant regression) is shown in Table 2. Out of 11 tumors resistant to irradiation 10 proved resistant to platidiam as well. On the other hand, only 10 out of 15 tumors resistant to platidiam were similarly resistant to radiation (Table 2). Tumors with combined resistance to radiation and platidiam were few - only 4 out of 20 (Table 2).

In analyzing the results, we should note the following. Radiotherapy in combination with chemotherapy, or chemoradiotherapy in combination with surgical intervention, and, in neglected cases, each of these treatment modalities alone are widely used in the treatment of lung cancer [9]. However, there is always a group of patients resistant to such combined treatment. This may be due to the combined resistance of tumor cells to the agents indi-

TABLE 1. Effects of Radiation and Platidiam on Human Pulmonary Carcinoma Transplants under the Renal Capsule of Animals

Case No.	Increment in control, rel. Un	Effect, %	
		radiation	platidiam
	Squamous-cell carcinoma		
1	0.38±0.08	10.0±0.2^{R}	2.0±0.2^{R}
2	0.28±0.06	122.0±2.7^{S}	24.0±0.9^{GI}
3	0.35±0.12	137.0±4.6^{S}	34.0±0.5^{GI}
4	0.68±0.76	40.0±1.5^{R}	5.0±0.7^{R}
5	1.00±0.90	69.0±5.3^{GI}	62.0±1.0^{GI}
6	0.26±0.12	25.0±0.3^{R}	23.0±2.7^{GI}
7	0.18±0.21	155.0±0.5^{S}	93.0±0.6^{GI}
8	0.42±1.00	79.0±0.6^{GI}	204.0±12.0^{S}
9	0.55±0.19	120.0±4.2^{S}	48.0±2.0^{R}
10	0.50±0.78	27.0±1.0^{R}	21.0±0.8^{R}
11	1.20±1.00	16.0±0.4^{R}	83.0±1.4^{GI}
12	0.72±0.57	20.0±1.3^{R}	14.0±0.6^{R}
13	0.20±0.52	205.0±14.0^{S}	40.0±4.3^{GI}
	Small-cell carcinoma		
14	1.00±0.89	130.0±7.1^{S}	190.0±17.0^{S}
15	0.27±0.10	36.0±1.6^{R}	23.0±0.4^{GI}
	Adenocarcinoma		
16	0.58±0.25	129.0±9.0^{S}	20.0±5.4^{GI}
17	0.50±0.69	110.0±1.3^{S}	24.0±1.5^{GI}
18	1.00±0.12	10.0±2.6^{R}	34.0±0.1^{GI}
19	1.00±3.50	8.0±0.7^{R}	73.0±5.0^{GI}
20	0.37±0.37	41.0±0.8^{GI}	259.0±11.0^{S}

Note. ^{R}: percent of transplant regression, ^{GI}: percent of transplant growth inhibition, ^{S}: percent of transplant growth stimulation.

TABLE 2. Comparison of Reaction of Pulmonary Carcinomas to Radiation and Platidiam in ST

Transplant reaction to radiation/ platidiam in ST	Number of cases
−/−	10
−/+	1
+/−	5
+/+	4

Note. Minus sign: tumor resistant to agent (platidiam or radiation) in ST; plus sign: tumor sensitive to agent (platidiam or radiation) in ST.

cated. Studies on transplanted cell lines have demonstrated the phenomenon of cross-resistance of tumor cells of some types to radiation and platinum agents [13]. Up to the present time it is not known to what degree the regularities revealed for transplanted cell lines may be extrapolated to human carcinomas.

Here we present the data of a parallel study of the reactions of pulmonary carcinoma transplants grown under the renal capsule of immunodepressed animals to platidiam and radiation. Radioresistance of tumors was found to be associated with their platidiam resistance in a high percentage of cases (in 10 out of 11). However, only 10 out of 15 tumors resistant to platidiam were resistant to radiation as well. This means that for some pulmonary carcinomas radioresistance correlates with platidiam resistance, a fact that provides indirect evidence of common mechanisms of chemo- and radioresistance functioning in primary human pulmonary carcinomas. At the same time, the fact that in some cases the tumors were resistant to platidiam but sensitive to radiation, as well as individual cases of tumor resistance to radiation in the presence of platidiam sensitivity, indicates that the chemo- and radioresistance of human carcinomas is governed not by one, but by several mechanisms of cell defense.

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