SENSITIVITY OF HETEROGRAFTED HUMAN MELANOMAS TO CHEMOTHERAPEUTIC AGENTS

M. V. Svyatukhin, V. L. Kovaleva, I. L. Sobol', and É. N. Malenkova

UDC 616-006.81-092.9-085.277.3

Experiments with explants of human melanomas cultivated in diffusion chambers in the peritoneal cavity of mice (10 cases) and explants into the retrobuccal pouches of golden hamsters (5 cases) revealed marked individual differences in the sensitivity of these tumors to combined treatment with three therapeutic preparations: vincristine (or vinblastine), nitrosomethylurea, and actinomycin D. Comparison of the results obtained in these two series of investigations showed agreement in three of five cases. In two cases the positive result obtained in the experiments with diffusion chambers corresponded to a negative result in the experiments on hamsters.

KEY WORDS: heterografted tumors; chemotherapy.

Heterografted human tumors constitute a type of experimental model useful for the study of the drug sensitivity of tumors in vitro, but preserving to some extent the possibility of interaction between the drug, the organism, and tumor. For instance, the method of heterografting of tumors into the retrobuccal pouches of hamsters has frequently been used in experimental chemotherapy [3, 4, 6-12]. As a rule these observations have been made on tumors subjected to prolonged passage. The writers know of only one investigation with primary transplanted tumors [3]. An advantage of experiments with the latter is that some idea can be obtained of the individual sensitivity of tumors in particular patients to the various compounds. Another type of heterografted human tumor consists of tumors transplanted into diffusion chambers. In the accessible literature the writers found only two papers whose authors had studied the action of several drugs on human tumors grown in diffusion chambers. In both cases tumors of the female reproductive organs were the test objects [5].

In this investigation the effect of a combination of three chemotherapeutic substances used in the clinic of the authors' institute on growth of heterografted human melanomas was studied.

EXPERIMENTAL METHOD

Melanoma tissue obtained from the operating theater from the Department of General Oncology, sent immediately in medium No. 199 with the addition of streptomycin and penicillin to the laboratory, was washed several times with the same medium and cut into small pieces, which were implanted into the retrobuccal pouches of golden hamsters weighing 100-150 g or placed one at a time into diffusion chambers, made from VUFS filters with a mean pore diameter of 0.23 μ, and implanted intraperitoneally into F_1(CBA × C57BL) mice weighing 28-45 g. The technique of the experiments with diffusion chambers was taken from Evgen'eva [1].

The therapeutic substances were injected intraperitoneally into the animals as a single dose in the following order: vincristine or vinblastine on the first day, nitrosomethylurea on the second day, and actinomycin D on the third day. Sterile pyrogen-free physiological saline was injected in the same doses

Institute of Experimental and Clinical Oncology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Blokhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 2, pp. 222-223, February, 1976. Original article submitted December 5, 1974.

TABLE 1. Mean Dimensions of Tumor Nodes 17-19 Days after Transplantation into Retrobuccal Pouches of Hamsters

Donor of tumor	Number of animals in group	Mean size of nodes (mm^2)		
		treated animals	untreated animals	P
K.	9	0,2	4,2	0,0025
R.	12	5,3	9,4	0,1
Shch.	9	5,1	6,2	0,1
G.	9	9,3	10,4	0,125
T.	6	4,6	3,9	0,25

and at the same time intraperitoneally into control animals. These injections into the hamsters were started 3-4 days after transplantation of the tumor, and into the mice when microscopic examination revealed signs of commencing growth of the graft, which happened 5-7 days or, less frequently, 12-14 days after implantation of the chambers. In one case the investigation began after some delay, namely 14 days after implantation of the chambers, when a wide radial zone of growth was already present. The substances were injected in the following doses: vincristine 0.15 mg/kg (hamsters) and 0.25 mg/kg (mice), vinblastine 1.5 mg/kg (hamsters) and 2.5 mg/kg (mice), nitrosomethylurea 38 mg/kg (hamsters) and 50 mg/kg (mice), and actinomycin D 133 μg/kg (hamsters) and 200 μg/kg (mice).

The tumor nodes in the hamsters attained their largest size 2-3 days after transplantation, after which they began to regress gradually. The only measurements made were two mutually perpendicular diameters of the node, and their product was determined. Toward the beginning of the therapeutic course this product varied between 6.6 and 15.7 mm^2 in different experiments.

Before the beginning of treatment, pairs of hamsters for the experimental and control groups were chosen with tumor nodes of about the same size. The results of measurement were assessed by Student's criterion for two-group analysis of covariance [2].

In the experiments with diffusion chambers the results were assessed on the basis of the pictures found on microscopic examination of the grafts at different times (5, 7, 10, 14, and 21 days after the end of the course of treatment); 5 or 6 grafts were studied at each time.

EXPERIMENTAL RESULTS

The data given in Table 1 show that much more rapid regression of the tumor nodes in the treated hamsters than in the control was observed in only one of the five cases.

In the experiments with diffusion chambers no difference in the rate of growth of the explants in the treated and control animals could be found in four of 10 cases. In the other six cases, on the other hand, a more or less marked delay in growth of the grafts was found in the animals receiving the therapeutic agents and this was interpreted as a positive result of chemotherapy. In four of these six cases, either no sign of growth of the explants could be seen in the treated animals during the 10-20 days after the

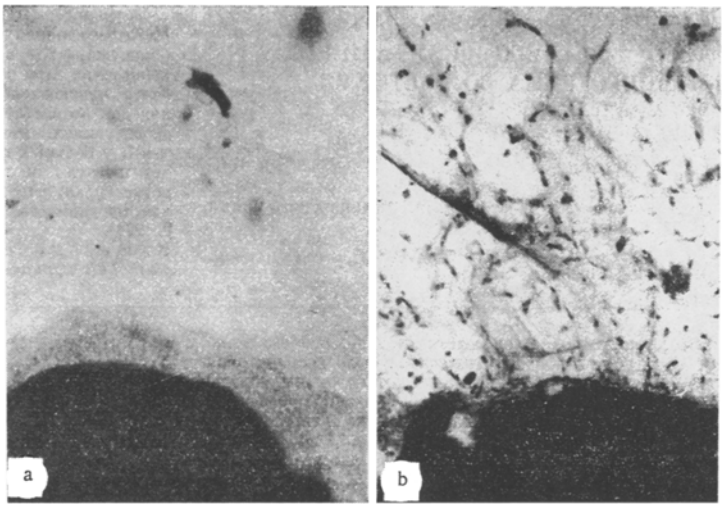

Fig. 1. Explanted tumors in diffusion chamber 5 days after end of administration of therapeutic agents: a) absence of growth of explants in "labeled" animals; b) wide radial zone of growth in control. Carazzi's hematoxylin, 90×.

beginning of the therapeutic course, or only very slight cellular outgrowths were found at the end of this period. Meanwhile in the control rapid growth was observed as early as after 5 days. In one case in this series, 7 days after the end of treatment only isolated cellular outgrowths were found in the mice, whereas in the control there was an extensive sheet of tumor cells. In another experiment 5 days after the end of the therapeutic course no sign of growth of the explants was found in the mice, whereas in the control there was a wide zone of growth (Fig. 1).

This account shows that the response of heterografted melanomas from different patients to the combination of chemotherapeutic agents tested had marked individual differences. The results obtained with grafts in diffusion chambers and in the retrobuccal pouches of hamsters agreed in three of five cases in which such comparison was possible. The divergent results obtained in two cases in the diffusion-chamber experiments revealed the sensitivity of the explants to the therapeutic agents, whereas no such sensitivity was found in explants of the same tumors grown in the retrobuccal pouches. On the basis of comparison of the two methods the writers are inclined to prefer heterografting of the tumor into diffusion chambers as the technically easier and more reliable method.

LITERATURE CITED

1. T. P. Evgen'eva (T. P. Evgenjeva), Europ. J. Cancer, 6, 533 (1970).
2. V. Yu. Urbakh, Biometric Methods [in Russian], Moscow (1964).
3. G. N. Friedel et al., Cancer (Philadelphia), 14, 1117 (1961).
4. R. Hertz, in: Choriocarcinoma (conference), Berlin (1967), pp. 26-32.
5. U. Heckman, Dtsch. Med. Wschr., 92, 932 (1967).
6. J. L. Lewis et al., Cancer Res., 29, 1988 (1969).
7. W. B. Paterson, Acta Un. Int. Cancer, 16, 714 (1960).
8. W. B. Paterson, Cancer Res., 28, 1637 (1968).
9. G. B. Piers et al., Cancer Res., 22, 563 (1962).
10. J. M. R. Smith, Brit. J. Cancer, 23, 78 (1969).
11. S. Sommers et al., Cancer (Philadelphia), 19, 674 (1966).
12. S. Sommers et al., Proc. Soc. Exp. Biol. (New York), 123, 740 (1966).