CHANGES IN PROLIFERATIVE ACTIVITY OF HUMAN BREAST CANCER CELLS CULTURED IN DIFFUSION CHAMBERS *in vivo* UNDER THE INFLUENCE OF ANTITUMOR PREPARATIONS

V. I. Shamaev, T. V. Krutova,
R. F. D'yachkovskaya, and D. B. Korman

UDC 613.33-006.6-018.15-092.4

KEY WORDS: diffusion chambers; breast cancer; labeling index; mitotic index; antitumor preparations.

An important role in the development of tumor chemotherapy is played by the study of the effect of preparations on proliferation of tumor cells. The limited amount of data of this sort for human tumors is due to the difficulty of conducting such investigations on patients, because many of them require repeated taking of tumor tissue, the use of radioactive isotopes, and so on. By culturing human tumor tissues in duffusion chambers (DC) *in vivo* it is possible to study the action of preparations under near-real conditions [7, 8], and also to study changes in the parameters of cell proliferation at various times after administration of antitumor agents. A combination of tumor tissue culture in DC *in vivo* with autoradiography is used. The action of preparations on proliferation is assessed by changes in the labeling index (LI), the mitotic index (MI), and the mean number of silver grains per nucleus (n) relative to the control, in % [4].

The object of the present investigation was to study the effect of thiophosphamide (thioTEPA), nitrosomethylurea (NMU), and diazan (1,2-bis-diazocetylethane) on proliferation of human breast cancer (BC) cells.

EXPERIMENTAL METHOD

Tumor tissue obtained from four patients with BC during operations was used. Two or three pieces of tumor, with a volume of 0.5-1 mm^3, were placed inside each DC (filter pore size 0.23 μ). Under hexobarbital anesthesia (200 mg/kg) 2 DC were implanted into the peritoneal cavity of each of a group of female CBA mice weighing 20-28 g. In each experiment 60-80 DC were used, and at each time point of measurement there were 4-6 DC. On the 3rd-7th day of culture the animals were given a single intraperitoneal injection, in the maximal tolerated doses, of thiophosphamide (12 mg/kg), NMU (100 mg/kg), and diazan (750 mg/kg). ^{3}H-thymidine (3.7×10^4 bq) was injected intraperitoneally into the mice 3.5, 24, 48, and 72 h after injection of the preparations, and the mice were killed 1 h later by cervical dislocation. Autoradiographs of total preparations of the cultures were prepared in the usual manner. LI of each tumor also was determined in vitro by incubation with ^{3}H-thymidine.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that all the tumors studied were characterized by low values and wide scatter of LI *in vitro*. Other workers also have reported similar variability [9, 10].

On the 7th day of culture in DC, LI was much higher than in vitro, probably due to stimulation of proliferation of the tumors in DC. Similar stimulation in DC has also been found for human gastric carcinoma cells [3]. Further supporting evidence is given by the high values of MI, because human BC in general is characterized by a low MI [2, 11]. The intensity of incorporation of ^{3}H-thymidine in the control also was high.

It will be clear from Fig. 1 that when NMU was given, LI fell after only 3.5 h to 15.6%, MI to 6.0%, and n to 15.9% compared with the control. These indices remained at the same level after 24 h. By 72 h some increase was observed in LI and n — to 48 and 29.8% respectively, but there was virtually no increase in MI.

Sector of Kinetics of Chemical and Biological Processes, Institute of Chemical Physics, Academy of Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 7, pp. 77-79, July, 1981. Original article submitted December 8, 1980.

0007-4888/81/9207- 0948$07.50

TABLE 1. Parameters of Proliferation of Human BC Cells in Vitro and on 7th Day of Culture in DC *in vivo* (mean values and limits of variations)

Histological structure of tumor	LI in vitro, %	In DC in vivo		
		LI, %	MI, %	n
Glandular carcinoma	0,8 (0,5—1,0)	17,6 (14,5—22,8)	4,4 (3,1—5,4)	31 (26—36)
Glandular carcinoma	3,7 (1,0—8,0)	29,1 (17,8—38,2)	4,0 (2,0—5,6)	54 (49—61)
Tubular carcinoma	4,2 (1,0—8,0)	23,4 (18,0—31,8)	3,7 (2,8—4,2)	57 (43—63)
Solid carcinoma	3,7 (1,0—7,0)	27,4 (22,8—34,0)	4,4 (2,8—5,6)	43 (38—52)

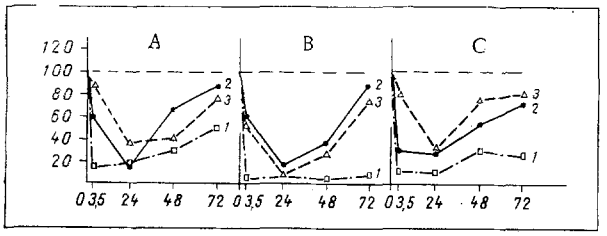

Fig. 1. Changes in LI (A), MI (B), and n (C) in human BC cells after a single injection of NMU (1), diazan (2), and thiophosphamide (3) (mean data of four experiments). Abscissa, time after injection of preparations (in h); ordinate, indices: in % of control).

Under the influence of thiophosphamide the indices of proliferation were lowered by a lesser degree and more slowly, the greatest decrease being found after 24 h (LI 37.7%, MI 7.8%, and n 31.7%). By 72 h the indices were close to the control level (77.9, 75.1, and 90.8%). The action of diazan on these parameters was similar to that of thiophosphamide. Maximal suppression of proliferation also was observed after 24 h (LI 14.3%, MI 16.3%, and n 24.1%). By the end of the experiments the parameters had risen to 86.5, 86.8, and 67.8% respectively.

Definite differences are thus observed in the action of these preparations on proliferation of human BC cells cultured in DC in vivo. NMU depressed proliferative activity abruptly and strongly, immediately after injection, and this inhibition of proliferation lasted throughout the experiment. The maximal effect of diazan and thiophosphamide was exhibited toward the end of the 1st day, and after 72 h proliferation had almost completely recovered.

On the basis of these results some hypotheses can be put forward to explain the effect of these compounds on the mitotic cycle of human BC cells. The low values of LI and n in the first few hours after injection of NMU can be regarded as an indication of considerable depression of the intensity of DNA synthesis and, possibly, of inhibition of the $G_1 \rightarrow S$ transition. The low MI during the first 3.5 h after injection of NMU was evidently the result of considerable lengthening of the G_2 phase. Similar results were obtained by a study of the effect of NMU on mammary gland tumor cells from C3H mice [5].

The appearance of the curves characterizing the action of thiophosphamide suggests that this compound had only a slight effect on the $G_1 \rightarrow S$ transition, for LI was virtually unchanged during the first 3.5 h. The considerable decrease in the intensity of DNA synthesis (n) and in the number of DNA-synthesizing cells (LI) after the first 24 h can be regarded as the result of the action of the compound on cells in the S phase.

Diazan also led to inhibition of DNA synthesis in the first few hours after injection. Comparison of curves showing changes in the number of DNA-synthesizing cells and the number of grains of silver under the influence of diazan emphasizes the fact that until 24 h LI continued to decline but the number of grains was unchanged. The reason may be that diazan inhibits the $G_1 \rightarrow S$ transition. Similar data were obtained by a study of the effect of diazan on the cell cycle of leukemia L-1210 [1]. The marked decline in MI immediately after injection of diazan points to inhibition of the cells in the G_2 phase also.

It can be concluded from these data that human BC cells are sensitive to all three compounds. The fact that diazan has similar activity to that of thiophosphamide, which is one of the most effective preparations for the treatment of BC, points to the desirability of a

clinical study of diazan for the treatment of this tumor. The deep and prolonged suppression of cell proliferation produced by NMU necessitates a revision of the view that NMU is ineffective against BC, which incidentally is based on only a small number of observations [6]. The study of the efficacy of NMU in the combined chemotherapy of BC will evidently also be fruitful.

LITERATURE CITED

1. S. A. Goncharov, N. P. Konovalova, and V. N. Shevtsova, Vopr. Onkol., No. 2, 68 (1976).
2. L. M. Ermolenko, Arkh. Patol., No. 8, 50 (1979).
3. T. V. Krutova, "Autoradiographic study of human gastric carcinoma," Author's Abstract of Candidate's Dissertation, Chernogolovka (1977).
4. V. I. Shamaev, T. V. Krutova, R. F. D'yachkovskaya, et al., in: Current Problems in Experimental Tumor Chemotherapy [in Russian], Chernogolovka (1980), Vol. 2, pp. 175-178.
5. N. M. Émanuél', D. B. Korman, L. A. Ostrovskaya, et. al., in: Nitrosoalkylureas – A New Class of Antitumor Preparations [in Russian], Moscow (1978).
6. N. M. Émanuél', E. M. Vermel, L. A. Ostrovskaya, et al., Cancer Chemother. Rep., 58, No. 2, 135 (1974).
7. U. Heckmann, Dtsch. Med. Wschr., 92, 932 (1967).
8. H. Limburg, in: Aktuelle Probleme der Therapie maligner Tumoren, Stuttgart (1973), pp. 7-16.
9. R. B. Livingston, U. Ambus, S. L. George, et al., Cancer Res., 34, 1376 (1974).
10. A. Sulkes, R. B. Livingston, and W. K. Murphy, J. Natl. Cancer Inst., 62, 513 (1979).
11. R. C. Young and V. T. De Vita, Cell Tissue Kinet., 3, 285 (1970).

CHANGES IN ACTIVITY OF OXIDATIVE ENZYMES IN CBA MOUSE THYMUS CELLS DURING CARCINOGENESIS INDUCED BY SIMIAN ADENOVIRUS SA7(C8)

A. I. Ageenko, G. G. Avtandilov, I. S. Kruglova, and I. K. Sviridova

UDC 616-006.6-02:578.827.22/-092.9-07:616.438-008.931:577.152.1

KEY WORDS: thymus; induced carcinogenesis; microspectrophotometry; activity of oxidative enzymes.

The thymus plays the leading role in the formation of the functional properties of the T lymphocytes and their immunocompetence. The least studied aspect of this problem at present is metabolism in the immunocompetent cells at the time of immunologic transformations [8]. The results of biochemical and histochemical studies of the thymus under normal conditions in animals of different species and in man have proved contradictory. The results of histochemical investigations of the thymus published in the literature are mostly based on visual estimate of activity of hydrolytic and oxidative enzymes [5, 7, 12]. There have been only isolated reports of changes in metabolic activity of thymocytes in different functional zones of the thymus during carcinogenesis [4].

The object of the present investigation was a microspectrophotometric study of changes in oxidative enzyme activity in mouse thymus cells in the postnatal period and during carcinogenesis induced by simian adenovirus SA7(C8), from the time of introduction of the virus until the period of progressive growth of the tumor.

EXPERIMENTAL METHOD

CBA mice were used. Simian adenovirus SA7(C8), in a titer of $10^{-4.5}$ CPD_{50}/ml, was used to induce the tumor and was injected subcutaneously into newborn animals during the first 24 h after birth in a dose of 0.1 ml. A tumor appeared in 95% of animals after 60 ± 4.5 days

Laboratory of Virology, P. A. Gertsen Moscow Oncologic Research Institute. Department of Pathological Anatomy and Laboratory of Medical Cytology, Central Postgraduate Medical Institute, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yannikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 7, pp. 80-82, July, 1981. Original article submitted August 4, 1980.

0007-4888/81/9207- 0950$07.50