DOI 10.1007/s10517-015-3103-6
Bulletin of Experimental Biology and Medicine, Vol. 160, No. 1, November, 2015

ONCOLOGY

Effect of Paclitaxel on Antitumor Activity of Cyclophosphamide: Study on Two Transplanted Tumors in Mice

V. I. Kaledin, V. P. Nikolin, N. A. Popova*, I. A. Pyshnaya, L. A. Bogdanova***, and T. S. Morozkova*****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 160, No. 7, pp. 93-95, July, 2015
Original article submitted April 8, 2014

Antitumor effect of paclitaxel used as the monotherapy or in combination with cyclophosphamide was studied on CBA/LacSto mice with transplanted LS and RLS tumors characterized by high (LS) and low (RLS) sensitivity to cyclophosphamide. The therapeutic effects of cyclophosphamide and paclitaxel were summed in animals with drug-resistant RLS tumor, while combined use of these drugs in LS tumor highly sensitive to the apoptogenic effect of cyclophosphamide was no more effective than cyclophosphamide alone.

Key Words: *malignant tumors; cyclophosphamide; paclitaxel; apoptosis*

Polychemotherapy is a common method for the treatment of malignant tumors. Drug combinations highly effective towards this or that tumor type are used [3]. However, despite satisfactory general statistic, there are always marginal variants of more or less effective treatment. This can be explained by individual characteristics of the tumor and carrier (deviations in activities of enzymes metabolizing this or that drug, their release from the cells, *etc.*), which prevent the realization of polychemotherapy effects. One of these characteristics is the capacity of some tumors to respond to certain drugs by programmed cell death. Radical cure can be attained in cases with these tumors. This positive result is now attributed to polychemotherapy or it is not attained, as insufficient dose of the apoptogenic drug in the drug combination prevents the manifestation of this effect. Other components of the combination are essential for activity of the apoptogenic drug, as they can modify its metabolism or release from cells or compete for intracellular targets, *etc.* For example, glucocorticoid hormones inducing apoptotic death of blood lymphocytes [5] induce resistance of epithelial tumor cells to drug-induced apoptotis [6] if used as components of a polychemotherapeutic composition. Presumably, other compounds can cause similarly unexpected effects [4,7].

We studied the effects of a new drug paclitaxel (multiple drug resistance system substrate, leading tumor cells to apoptotic death) used as monotherapy or in combination with cyclophosphamide (CP) on two experimental tumor models differing by sensitivity to the apoptogenic activity of CP.

MATERIALS AND METHODS

The study was carried out on 3-month-old CBA/LacSto (CBA) male mice (n=65) from vivarium of Institute of Cytology and Genetics. Three experimental series

Institute of Cytology and Genetics, Siberian Division of the Russian Academy of Sciences; *Novosibirsk State University; **Institute of Chemical Biology and Basic Medicine, Siberian Division of the Russian Academy of Sciences; ***Institute of Molecular Pathology and Pathomorphology, Novosibirsk, Russia. ***Address for correspondence:*** pathol@inbox.ru. V. I. Kaledin

were carried out. The mice were kept 6-9 per cage at artificial illumination (12:12 h day:night) with free access to water and fodder. All manipulations on animals were carried out in accordance with the international regulations (86/609/EEC).

Previously derived transplantable lymphoid tumor strains LS and RLS with high (LS) and low (RLS) sensitivity to apoptogenic and antitumor effects of CP served as the tumor models [1,2].

Tumor growth was induced by transplantation of 10^6 LS or RLS cells washed from ascitic fluid in 0.1 ml saline. The cells were injected into the right hip. After tumor appearance, it was measured with a caliper and the mice were distributed into groups by the results of measurements. The treatment was carried out on day 10 after RLS tumor cell transplantation and on day 13 or 14 after LS tumor transplantation.

CP (DEKO) and paclitaxel-LENS (LENS-Pharm) were injected into the abdominal cavity in a single dose in 1 ml saline/100 g.

The animals with RLS tumor were injected with CP in a dose of 100 mg/kg, with LS tumor – 30 or 15 mg/kg; paclitaxel dose was 30 mg/kg in all cases. Mice were regularly weighed; the tumors were measured with a caliper. The animals were decapitated after the tumors reached the size of about 3 cm^3. The tumors were resected and weighed.

The data were statistically processed by common methods, the significance of differences between the means was evaluated by Student's *t* test.

RESULTS

Tumor growth was arrested within 2 days after injection of paclitaxel on day 14 after transplantation of LS tumor; tumor growth then restarted and after 2 more days the tumor was 2-fold larger than initially (Table 1). The tumors continued to grow during 24 h after CP injection, after which they rapidly shrank till complete regression (Table 1). These results suggested

TABLE 1. Effects of Paclitaxel and CP Injected Separately in a Single Dose of 30 mg/kg on LS Tumor Size (cm^3) in Male CBA Mice

Day after injection	Paclitaxel	CP
0	1.4±0.17	1.2±0.17
1	1.3±0.17	2.0±0.10**
2	1.4±0.19	0.8±0.09*
5	3.5±0.86*	0

Note. *p<0.05, **p<0.01 in comparison with day 0. Each group consisted of 5 animals.

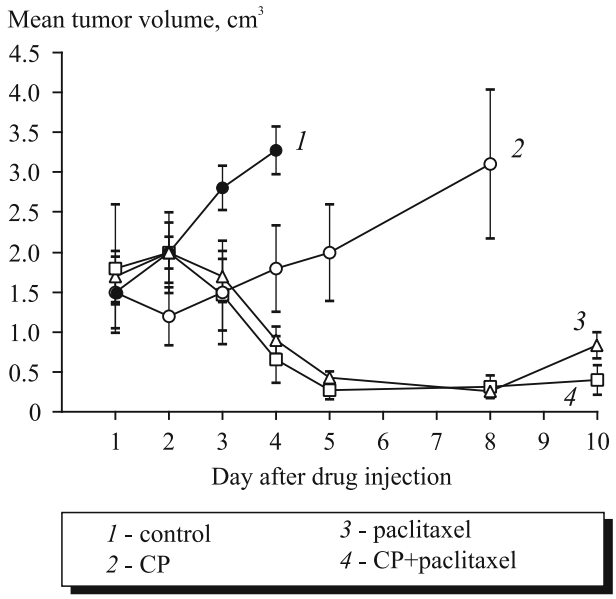

Fig. 1. Time course of LS tumor growth after intramuscular transplantation in male CBA mice.

reducing CP dose to 15 mg/kg in the next experiment (evaluation of the effects of combined paclitaxel+CP effects on LS tumor) with monotherapy (each drug) control groups.

Tumor growth was somewhat inhibited 24 h after paclitaxel injection, but then restarted, though slower (Fig. 1) than in control; tumor growth was 43% inhibited 4 days after the injection. Tumor growth continued during 24 h after CP injection, similarly as in the previous experiment, but later the tumors started to regress, as a result of which tumor growth was almost 72% inhibited after 4 days. Tumor volume was stable during 3 days more, after which it increased. The time course of tumor growth (and regression) in mice receiving CP+paclitaxel was largely the same as in animals receiving CP monotherapy (Fig. 1, curves *2* and *3*). However, the toxicity of the drugs was more severe in this case. Only 1 of 5 animals injected with paclitaxel alone died, *vs.* 3 of 9 animals in combined therapy (on days 5, 6, and 7).

All animals developed RLS tumors on day 10 after transplantation (Fig. 2). The animals were divided into 4 groups receiving different therapies. Group 1 mice (control) received no treatment. Group 2 received CP (100 mg/kg). Group 3 received paclitaxel (30 mg/kg). Group 4 animals received CP and paclitaxel in the same doses simultaneously. The mean tumor volume in the groups was 0.16±0.01 cm^3. Body weights of mice changed negligibly during the first days after CP or paclitaxel monotherapies. After combined treatment with the two drugs, the body weights decreased by 6.3% over 4 days in comparison with the initial weight. Tumor growth was inhibited similarly during 3-4 days after

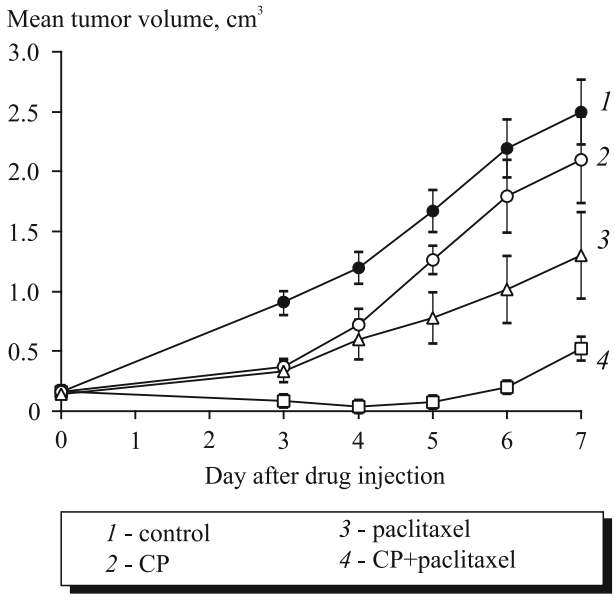

Fig. 2. Time course of RLS tumor growth after intramuscular transplantation in male CBA mice.

monotherapy. Later the tumors in mice injected with CP were growing at the same velocity as in controls and slower in animals injected with paclitaxel (50% inhibition over the entire period of observation).

The tumors could be hardly palpated during the first 4 days after combined therapy in all animals, and reached the initial size (about 0.2 cm^3) only on day 6. Later the tumors were growing with the same velocity as in animals injected with paclitaxel alone. Importantly that paclitaxel monotherapy caused no deaths from toxicity, while combined paclitaxel and CP treatment led to death of 3 of 5 mice (on days 4, 6, and 7 after injection).

Hence, the therapeutic effects of CP and paclitaxel were summed up or even synergic in animals with RLS tumors; this suggested combined use of these drugs in tumors of this type. Importantly that paclitaxel, causing a 50% growth inhibition in LS tumors, was virtually inessential for the effect of CP, which was more active in this antitumor combination. It seems that CP-induced subtotal apoptosis of cells in this tumor prevented cell death which could have developed under the effect of paclitaxel; this made useless (and, judging by increase of toxicity, harmful) the use of this drug combination in LS tumor. On the other hand, our results and a previous report [2] indicated that a higher dose of an apoptogenic drug could inhibit the tumor growth more intensely and lead to radical cure; hence, these tumors should be detected before treatment.

REFERENCES

1. V. I. Kaledin, V. P. Nikolin, T. A. Ageeva, *et al.*, *Vopr. Onkol.*, **46**, No. 5, 588-593 (2000).
2. V. I. Kaledin, N. A. Popova, and E. M. Andreeva, *Vopr. Onkol.*, **52**, No. 1, 70-73 (2006).
3. *Manual for Chemotherapy for Malignant Tumors* [in Russian], Moscow (2000).
4. N. Casares, M. O. Pequignot, A. Tesniere, *et al.*, *J. Exp. Med.*, **202**, No. 12, 1691-1701 (2005).
5. B. F. Gruber, R. Sgonc, Y. H. Hu, *et al.*, *Eur. J. Immunol.*, **24**, No. 5, 1115-1121 (1994).
6. I. Herr, E. Ucur, K. Herzer, *et al.*, *Cancer Res.*, **63**, No. 12, 3112-3120 (2003).
7. E. Vacchelli, L. Galluzzi, W. H. Fridman, *et al.*, *Oncoimmunology*, **1**, No. 2, 179-188 (2012).