# Potentiated Cyclophosphane: Experimental Study of the Effect on Tumor Development and Efficiency of Cytostatic Therapy

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Experiments on animals with transplanted tumors (Lewis lung carcinoma and carcinosarcoma Walker-256) showed that combination treatment with cyclophosphane and its homeopathically potentiated forms increases antiblastic activity of the preparation.

**Key Words:** *transplanted tumors; cytostatic; potentiated forms*

Chemotherapy of tumors suggests the use of various preparations. Depending on the chemical structure and mechanism of action, they are divided into groups of alkylating compounds, antimetabolites, antibiotics, plant substances, platinum-containing agents, *etc*. [3,6]. Despite much progress in drug therapy of tumors, the search for new preparations that would increase the efficiency of treatment in patients with malignant neoplasms is an urgent problem.

Our previous studies showed that homeopathically potentiated substances in ultralow doses possess pharmacological activity [5,8,9]. It is important that potentiated substances increase the efficiency and reduce toxicity of medicinal preparations during combination therapy with molecular (therapeutic) and ultralow doses. This phenomenon receiving the name "bipathy" is considered to be universal [5]. Here we studied the effect of potentiated cyclophosphane (PCP) on antitumor activity of the cytostatic.

## MATERIALS AND METHODS

PCP was prepared by homeopathic potentiation and administered in dilutions C12, C30, and C200, which corresponded to concentrations of $10^{-24}$, $10^{-60}$, and $10^{-400}$ wt %, respectively ($PCP_{30}$; $PCP_{200}$; and MIXT, $PCP_{12+30+200}$).

Experiments were performed on 190 C57Bl/6 mice and 44 female outbred rats with transplanted tumors. The animals were obtained from the Laboratory of Experimental Biological Modeling (Institute of Pharmacology, conventional strain). Lewis lung carcinoma was transplanted intramuscularly into the hindlimb thigh of mice ($4\text{-}6\times10^6$ cells in 0.1 ml physiological saline). Carcinosarcoma Walker-256 was transplanted subcutaneously to rats by routine methods (20% tumor cell suspension in 0.2 ml physiological saline) [7].

During cytostatic therapy the mice intraperitoneally received cyclophosphane (CP) in a single dose of 125 mg/kg 10-13 days after transplantation of tumor cells. The rats intraperitoneally received cyclophosphane in a dose of 20 (3 injections at 48-h intervals) or 60 mg/kg (single treatment); the therapy started 14 days after tumor transplantation.

PCP or solvent (distilled water; C30, C200, or $C_{12+30+200}$) was administered intraperitoneally or perorally in doses of 0.2 ($PCP_{30}$), 0.3 (PCP200 and $PCP_{12+30+200}$, mice), and 0.4 ml (rats) 1 h after injection of the cytostatic. Then the preparation was given for 8-10 days.

The efficiency of therapy was evaluated on days 19-23. The weight of tumors and index of tumor growth suppression (TGS) were estimated. The intensity of metastatic dissemination was evaluated. We determined the count and diameter of lung metastases (individual and average values). The area of metastases was calculated by the formula $\pi r^2$. The incidence of metastatic dissemination was calculated as the ratio between the number of animals with metastases and total count of animals in the group (%). The index for inhibition of metastasizing (IIM) reflecting the degree of metastatic lesions was calculated by the formula [2]:

$$IIM=[(A_C\times B_C)-(A\times B)]\times100\%/[A_C\times B_C],$$

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**TABLE 1.** Effect of Intraperitoneally Injected $CP_{30}$ on the Development of Lewis Lung Carcinoma and Efficiency of Chemotherapy in Female C57Bl/6 Mice ($\bar{X}\pm m$)

| Parameter | Control | | CP | | CP+$PCP_{30}$ | | $PCP_{30}$ |
|---|---|---|---|---|---|---|---|
| | series I ($n$=11) | series II ($n$=15) | series I ($n$=10), day 10 | series II ($n$=10), day 10 | series I ($n$=10) | series II ($n$=15) | series I ($n$=10) |
| Tumor weight, g | 6.35±0.25 | 7.56±0.20 | 4.48±0.39* | 5.79±0.27* | 4.40±0.32 | 6.15±0.21* | 6.27±0.32 |
| TGS, % | | | 29 | 23 | 31 | 19 | 1 |
| Incidence of metastatic dissemination, % | 100 | 100 | 80* | 90 | 50+ | 53+ | 100 |
| Number of metastases | 23.45±3.59 | 22.73±3.59 | 5.70±1.16* | 4.10±1.28* | 2.90±1.12* | 1.07±0.40* | 18.90±2.60 |
| Area of metastases, $mm^2$ | 28.05±4.04 | 19.70±4.04 | 4.14±3.69* | 1.69±0.72* | 0.45±0.29* | 0.33±0.21* | 38.75±10.90 |
| IIM, % | | | 81 | 87 | 94 | 98 | 19 |

**Note.** Series II was performed to confirm the results of series I (reproducibility of the effect). Here and in tables 2-4: $p<0.05$: *compared to the control; +compared to the CP group. $n$, number of animals.

where $A_C$ and $A$ are the incidence of metastatic dissemination to the lungs in control and experimental mice, respectively; and $B_C$ and $B$ are the average number of lung metastases in control and experimental animals, respectively.

Carcinosarcoma Walker-256 is characterized by hematogenous and lymphatic dissemination. By the end of experiments we determined the weight of axillary lymph nodes with metastatic lesions.

The results were analyzed by nonparametric Mann-Whitney $U$ test and Fischer's angular transformation ($\varphi$) [4].

## RESULTS

In series I intraperitoneal injection of $PCP_{30}$ had no effect on tumor growth in mice with Lewis lung carcinoma (Table 1). Combination treatment with CP and $PCP_{30}$ did not change tumor weight. However, the test preparation increased antiblastic activity of the antitumor agent. Metastases were found in 80% animals receiving the cytostatic. Metastatic lesions in the lungs were revealed only in 50% mice treated with $PCP_{30}$ and CP. IIM was highest in animals receiving combination therapy. In these mice the number and area of metastases decreased by 1.9 and 9.2 times, respectively, compared to the control (Table 1).

In series II $PCP_{30}$ potentiated the antimetastatic effect of CP. After the course of treatment with $PCP_{30}$ and cytostatic the number of animals with lung metastases decreased to 53%. However, metastases were found in 90% mice receiving only CP (Table 1). Cytostatic therapy did not decrease the incidence of tumor dissemination, but reduced the number (by 5.5 times) and area of lung metastases (Table 1). After combination therapy with $PCP_{30}$ and CP the number and area of lung metastases were lower than in cytostatic-receiving mice by 3.8 and 5.1 times, respectively. It should be emphasized that 4 of 14 mice treated with CP died (29%, $P\varphi<0.001$). We did not observe death of animals from other groups.

**TABLE 2.** Effect of $PCP_{200}$ on the Development of Lewis Lung Carcinoma and Efficiency of Chemotherapy in Female C57Bl/6 Mice ($\bar{X}\pm m$)

| Parameter | Control ($n$=9) | CP ($n$=11) | $PCP_{200}$ ($n$=7) | CP+$PCP_{200}$ | |
|---|---|---|---|---|---|
| | | | | intraperitoneally ($n$=13) | perorally ($n$=12) |
| Tumor weight, g | 6.40±0.25 | 4.49±0.19* | 5.39±0.69 | 3.64±0.27* | 4.01±0.37 |
| TGS, % | | 30 | 16 | 43 | 37 |
| Incidence of metastatic dissemination, % | 100 | 64* | 100 | 36 | 25+ |
| Number of metastases | 20.67±4.03 | 1.55±0.71* | 11.29±5.02 | 0.36±0.20* | 0.50±0.26* |
| Area of metastases, $mm^2$ | 15.59±3.38 | 0.37±0.22* | 9.57±5.13 | 0.03±0.01* | 0.10±0.07* |
| IIM, % | | 95 | 45 | 99 | 99 |

**TABLE 3.** Effect of $PCP_{200}$ and $PCP_{12+30+200}$ on the Efficiency of Chemotherapy in Male C57Bl/6 Mice with Lewis Lung Carcinoma ($\bar{X}\pm m$, $n$=11)

| Parameter | Control | CP | +$PCP_{200}$ | +$PCP_{12+30+200}$ |
|---|---|---|---|---|
| Tumor weight, g | 6.11±0.70 | 2.30±0.35* | 2.46±0.31* | 3.36±0.24* |
| TGS, % | | 62 | 60 | 45 |
| Incidence of metastatic dissemination, % | 100 | 45* | 18 | 0+ |
| Number of metastases | 19.18±2.57 | 0.45±0.16* | 0.27±0.14* | 0 |
| Area of metastases, $mm^2$ | 8.48±2.15 | 0.03±0.01* | 0.02±0.01* | 0 |
| IIM, % | | 99 | 99 | 100 |

We studied the effects of intraperitoneal and peroral treatment with $PCP_{200}$ on the efficiency of cytostatic therapy in mice with tumors. $PCP_{200}$ markedly increased antimetastatic activity of CP (similarly to $PCP_{30}$, Table 2). The number of lung metastases in mice receiving combination therapy decreased. The number and area of metastases decreased after peroral (by 3.1 and 3.7 times, respectively) and intraperitoneal treatment with $PCP_{200}$ (by 4.3 and 12.3 times, respectively). We showed for the first time that intraperitoneal administration of $PCP_{200}$ increases antitumor activity of the cytostatic in relation to primary tumors. However, CP-produced changes in tumor weight were insignificant (Table 2). Therefore, $PCP_{200}$ retained pharmacological activity under various regimens of treatment.

In series III we compared the effects of $PCP_{200}$ and MIXT ($PCP_{12+30+200}$). These preparations were administered through a probe (less traumatic procedure). CP markedly suppressed tumor growth. The weight of primary tumors decreased by 2.7 times compared to the control. Only 45% animals receiving the cytostatic had lung metastases (Table 3).

Combination treatment with CP and $PCP_{200}$ or MIXT had no effect on tumor weight. PCP increased antimetastatic activity of the cytostatic (Table 3). Since MIXT displayed a greater antimetastatic activity during combination treatment with the cytostatic, further experiments were performed with this potentiated form.

We compared the effects of MIXT on the efficiency of treatment with CP in therapeutic and knockout doses in mice with carcinosarcoma Walker-256. The cytostatic in both doses markedly suppressed primary tumor growth. The course of treatment with CP in a dose of 20 mg/kg decreased tumor weight by 3.8 times. In rats receiving this cytostatic in a single dose of 60 mg/kg tumor weight was 3.1-fold lower than in control animals (Table 4).

CP prevented lymphatic dissemination of tumors cells. After treatment with the cytostatic in doses of 20 and 60 mg/kg metastatic lesions in the lymph nodes were found only in 20 and 25% rats, respectively (*vs.* 78% in the control). It should be emphasized that the course of treatment with CP in a dose of 20 mg/kg significantly increased the number of rats with hematogenous lung metastases.

**TABLE 4.** Effect of $PCP_{12+30+200}$ on the Efficiency of Cytostatic Therapy in Female C57Bl/6 Mice with Carcinosarcoma Walker-256 ($X\pm m$, $n$=8-10)

| Parameter | Control | CP, 20 mg/kg | | CP, 60 mg/kg | |
|---|---|---|---|---|---|
| | | without $PCP_{12+30+200}$ | with $PCP_{12+30+200}$ | without $PCP_{12+30+200}$ | with $PCP_{12+30+200}$ |
| Tumor weight, g | 79.2±14.3 | 20.9±2.2* | 9.9±2.9+ | 25.8±4.4* | 10.9±1.8+ |
| TGS, % | | 74 | 88 | 67 | 86 |
| Weight of axillary lymph nodes, mg | 825.3±278.4 | 225.9±51.8* | 177.9±20.7 | 191.8±17.7* | 196.6±14.2 |
| Number of lung metastases | 1.6±1.4 | 1.0±0.33* | 2.4±1.3 | 0.4±0.3 | 1.1±0.6 |
| Number of animals with metastases, % | | | | | |
| lymphatic | 78 | 20* | 0 | 25 | 13 |
| hematogenous | 22 | 60* | 67 | 25 | 38 |
| Total number of animals with metastases, % | 78 | 80 | 67 | 38 | 38 |

During combination treatment MIXT potentiated the antitumor effect of cyclophosphane. In rats receiving 20 mg/kg CP and MIXT tumor weight decreased by 8.0 times compared to the control. After administration of MIXT and cytostatic in a dose of 60 mg/kg tumor weight decreased by 7.3 times. Metastatic lesions in the lymph nodes were not revealed after the course of treatment with MIXT and 20 mg/kg CP. MIXT did not change antimetastatic activity of CP in a dose of 60 mg/kg (Table 4). Experiments on female rats indicate that MIXT potentiates the inhibitory effect of CP on primary tumor growth. It was observed after the course of treatment and single administration of this cytostatic.

Our findings indicate that the efficiency of CP therapy in mice with tumors markedly increases after combination treatment with potentiated forms. Previous experiments on mice with melanoma B-16 and lung cancer LC-67 revealed that CP has a greater antitumor activity during combination treatment with MIXT [1]. The increase in antimetastatic activity of the cytostatics produced by PCP is of particular interest. The duration of life in patients with malignant neoplasms depends on the degree of metastatic dissemination. Our results confirm high efficiency of bipathic therapy.

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