EFFECT OF THE RHYTHM OF CYCLOPHOSPHAMIDE ADMINISTRATION ON ITS ANTITUMOR ACTION AND THE IMMUNE RESPONSE OF MICE WITH METHYLCHOLANTHRENE SARCOMA

S. P. Gordienko,
V. M. Bergol'ts,
and K. V. Botsmanov

UDC 616-006.3.04-082.9-02:615.277.4]-085.277.3-07:616.15-097.5

The therapeutic effect of cyclophosphamide (CP) in relation to the dose and rhythm of its administration was studied in C57BL/6 mice with a methylcholanthrene-induced sarcoma. The optimal scheme giving a complete cure of the experimental animals was 5 injections, each of 199 mg/kg CP at intervals of 10 days. The effect of the total dose of CP on humoral antitumor immunity was demonstrated: after injection of 100 mg/kg CP the animals' serum lost its ability to stimulate tumor growth and acquired cytotoxic properties. The serum of animals receiving total doses of CP above 400 mg/kg lost its effect on tumor growth. The doses of CP used did not affect cellular antitumor immunity (the cytotoxicity of the lymphocytes and resistance of the tumor to retransplantation).

KEY WORDS: cyclophosphamide – rhythm of administration; methylcholanthrene sarcoma; cytotoxicity; antitumor immunity.

Despite the many experimental and clinical investigations into the effect of cyclophosphamide (CP) on the growth of malignant tumors there is still no general agreement regarding the optimal doses and rhythm of its administration [1-8]. The results of experiments on animals without tumors indicate that antitumor agents including CP, depending on the dose and rhythm of their administration, may considerably modify the immunologic reactivity of the organism [9, 10]. Meanwhile no data on the effect of the intervals and rhythm of CP administration on the immune response of tumor-bearing animals could be found in the accessible literature.

The object of the investigation was to study the effect of different rhythms of administration of CP on its antitumor action and on the immune response of mice with methylcholanthrene sarcoma.

EXPERIMENTAL METHOD

Inbred C57BL/6 mice were used. A polymorphocellular sarcoma was induced by 20-methylcholanthrene and its first 3 generations were used. To obtain serum the tumor-bearing mice were totally exsanguinated by division of the axillary vascular plexus. Lymphocytes were obtained from the peripheral lymph glands.

The cytotoxicity of the immune lymphocytes and serum in vivo against cells of a syngeneic tumor were tested by Winn's method [12]. In the first case living sarcoma cells (1×10^5) and immune or normal syngeneic lymphocytes were mixed in the ratio of 1 : 200. In the second case, the sarcoma cells were treated

Laboratory of Experimental Therapy of Tumors, P. A. Gertsen Moscow Oncologic Scientific-Research Institute, Moscow. Department of Pathomorphology, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 7, pp. 87-90, July, 1974. Original article submitted June 8, 1973.

with 0.15 ml immune or normal serum. After incubation at 37°C for 30 min the mixture was injected subcutaneously into intact syngeneic mice. After 10 days the mice were killed and the tumors weighed. The percentage inhibition of tumor growth (the effect of the lymphocytes or serum) was calculated by the formula

$$\frac{X_e - X_c}{X_c} \times 100,$$

where X_e is the mean weight of the tumor in the experimental group and X_c the mean weight of the tumor in the control group.

Antitumor resistance was tested by reinoculating the experimental mice with the transplantation dose of tumor cells (1×10^5) 35 days after complete regression of the tumors.

A cell suspension of the methylcholanthrene sarcoma (MCS) was obtained by trypsinization. The cells were counted by Schrek's method [11].

CP of East German manufacture was used. The compound was diluted in distilled water before use and injected intraperitoneally as a 0.2% solution. Depending on the dose and rhythm of CP administration the animals were divided into 7 groups (Table 1). The results were subjected to statistical analysis by the Student–Fisher method.

EXPERIMENTAL RESULTS

In the experiments of series I the antitumor action of CP was studied in C57BL/6 mice with MCS (2nd and 3rd generations) depending on the frequency of its administration and the total dose. CP was given in a dose of 100 mg/kg, which is only 40% of the maximal tolerated dose, but (judging by the increased length of survival of the mice with tumors) it has antitumor action equal to that of a dose of 250 mg/kg.

In the mice receiving CP in single doses of 100 and 250 mg/kg growth of the tumor was inhibited considerably and the life span of the mice with the tumors was increased, but a cure was not observed in any of the animals. Complete regression of the tumor was observed in 100% of cases of mice receiving CP as 5 separate injections at intervals of 10 days (total dose 500 mg/kg). No recurrence of the tumor took place during 120 days of observation.

Three injections of CP at the same intervals (total dose 300 mg/kg) also led to complete regression of the tumor in 100% of the experimental animals. Under these circumstances, however, the tumor recurred during the period of observation in 34% of the mice.

If the intervals between injections were increased to 15 days but the total dose kept at 500 mg/kg (5 injections), recurrences of the tumor took place in 40% of the animals.

In the tumor-bearing mice receiving CP at intervals of 3 days (total dose 300 mg/kg) regression of the tumor was observed in 100% of the animals that survived. With this scheme of CP injection, however, the mortality of the mice from the toxic action of the compound was very high (69.7%).

TABLE 1. Effect of Serum* of Tumor-Bearing Mice Treated with CP Injected Once every 10 Days (total dose 500 mg/kg) on Growth of MCS†

Group	Mice from which serum was obtained	Mean wt. of tumors in group (in mg)	Stimulation (+) or inhibition (–) of tumor growth (in %)	P
1	Intact (untreated)	73,2±2,7		
2	Tumor-bearing	115±5,8	+152	<0,001
3	Receiving CP once (100 mg/kg)	23,3±2,1	–68	<0,001
4	Twice (200 mg/kg)	11,1±1,94	–84	<0,001
5	Three times (300 mg/kg)	40,3±2,26	–45	<0,001
6	Four times (400 mg/kg)	76±3,08	+3,8	>0,5
7	Five times (500 mg/kg)	77±4,37	+6,3	0.5>P>0,2

*Serum taken from mice on 4th day after injection of CP.

†Sarcoma cells injected subcutaneously in the dorsal region in a dose of 1×10^5 with 0.1 ml of the test serum.

The effect of different doses and rhythms of CP administration on the immune response of the tumor-bearing mice was studied in the experiments of series II.

The results of these experiments showed (Table 1) that the serum of the untreated mice with tumors induced marked stimulation of tumor growth in the recipient mice. Meanwhile serum taken from tumor-bearing mice receiving CP injections once every 10 days had a marked cytotoxic action on the tumor cells. However, if the total dose of CP amounted to 400 and 500 mg/kg the serum of the mice no longer had any effect on tumor growth. Similar results were obtained in the experiments in which the compound was injected at intervals of 15 days.

After injection of CP once every 3 days the serum of mice receiving the compound in doses of 100 and 200 mg/kg had a cytotoxic action. Increasing the total dose of the compound to 300 mg/kg led to loss of the effect of the serum on tumor growth.

The experiments to study the cytotoxicity of the immune lymphocytes of the tumor-bearing mice showed that lymphocytes of the tumor-bearing mice had a cytotoxic action regardless of the rhythm of injection or the total dose of CP. In all groups the cytotoxic index was 1. Lymphocytes of intact mice had no effect on tumor growth.

Tumor resistance was next studied. For this purpose the mice of all groups were inoculated 35 days after complete regression of the tumors with a transplantation dose of cells (1×10^5) from a second-generation sarcoma.

The results of these experiments showed that reinoculation with sarcoma cells of C57BL/6 mice treated with CP, administered by various schemes, did not lead to development of a tumor.

The results of these experiments on syngeneic mice thus showed that by changing the interval between injections of the same sessional dose of the compound the therapeutic effect could differ in its strength, up to complete cure of the animals with tumors. The optimal scheme of treatment of MCS was 5 injections of CP at intervals of 10 days (total dose 500 mg/kg).

Investigation of serum obtained from mice treated with CP administered by different schemes showed that the compound modifies the properties of the serum considerably: it lost its ability to stimulate tumor growth and acquired cytotoxic properties, but after an increase in the total dose of CP to 400 or 500 mg/kg the serum no longer had any effect on tumor growth. This fact can evidently be explained on the grounds that in these doses the compound completely suppressed the humoral immune response.

The preservation of the cytotoxicity of the immune lymphocytes and resistance to reinoculation with MCS is evidence of the stability of the cellular factors of antitumor immunity toward the action of CP.

The results correlate with those obtained by Brondz [1], who showed that the lymphocytes of irradiated mice immunized with sarcoma MCh-1 preserve their cytotoxic properties at a time of complete absence of humoral antibodies against the same antigens in the body. In this respect the properties of antitumor and transplantation immunity are identical. In particular, it was shown that during repeated skin grafting cytotoxic agents do not increase the period of survival of the graft.

It seems very probable that CP and the cellular factors of immunity of the tumor-bearing animal act synergistically in their antitumor effect.

LITERATURE CITED

1. B. D. Brondz, Zh. Obshch. Biol., 27, No. 1, 80 (1966).
2. V. I. Vasil'eva, Byull. Éksperim. Biol. i Med., No. 2, 85 (1970).
3. A. M. Garin, V. I. Astrakhan, M. B. Bychkov, et al., Vopr. Onkol., No. 10, 3 (1965).
4. S. P. Gordienko, in: Papers on Clinical and Experimental Oncology [in Russian], Moscow (1971), p. 22.
5. S. P. Gordienko and K. V. Botsmanov, Éksper. Khir., No. 2, 50 (1972).
6. A. B. Syrkin and V. N. Solenov, Farmakol. i Toksikol., No. 6, 723 (1969).
7. A. B. Syrkin, V. N. Solenov, D. A. Bodyagin, et al., Vestn. Akad. Med. Nauk SSSR, No. 3, 55 (1971).
8. G. Mathe, I. Schneider, and L. Schwarzenberg, Europ. J. Cancer, 6, 23 (1970).
9. G. W. Santos, Fed. Proc., 26, 3 (1967).
10. R. S. Schwartz, in: Immunity, Cancer and Chemotherapy, New York (1967), p. 203.
11. R. Schrek, Am. J. Cancer, 28, 389 (1936).
12. H. J. Winn, J. Immunol., 86, 228 (1961).