# TREATMENT OF TRANSPLANTABLE MOUSE LEUKEMIA OF THE HEMOCYTOBLASTOSIS TYPE WITH CYCLOPHOSPHAMIDE IN CONJUNCTION WITH FREUND'S ADJUVANT

G. M. Sukhin, V. S. Ter-Grigorov, and V. M. Bergol'ts

UDC 616.155.392-092.9-085.
277.3-059 : 615.046

The effect of combined administration of cyclophosphamide and Freund's complete adjuvant was studied in experiments on C57BL/6 mice with a transplanted leukemia-hemocytoblastosis of the Puyman strain. The maximum therapeutic effect, shown by an increase in the mean life span of the mice from 11.7 days in the control to 106.4 days, and survival of 42.9% of the animals for more than 180 days was obtained when cyclophosphamide was given as a single dose of 250 mg/kg body weight on the 3rd day after transplantation of the leukemia with subsequent (7 days later) injection of Freund's adjuvant (and also after two such courses of treatment).

During recent years methods of chemotherapy of malignant tumors combined with activation of the immune forces of the tumor carrier by means of adjuvants or of bacterial preparations possessing adjuvant action have been intensively studied and developed [3, 7, 9, 11].

The object of the present investigation was to study the effectiveness of treatment of a transplantable mouse leukemia-hemocytoblastosis of Puyman's strain La by cyclophosphamide (CP) in conjunction with Freund's complete adjuvant (FCA).

## EXPERIMENTAL METHOD

Experiments were carried out on 210 C57BL/6 mice with transplantable leukemia-hemocytoblastosis. The animals were divided into groups with 20-30 mice in each group. CP was injected intraperitoneally on the 3rd day after transplantation of the leukemia in a dose of 250 mg/kg, once or twice at an interval of 17 days, alone or in various combinations with FCA.

The FCA (Difco, USA) was injected intraperitoneally in a dose of 0.1 ml per mouse daily either 3 days after transplantation of the leukemia, simultaneously with CP or 7 days after it. The animals of one group, after the first course of treatment which included injection of CP on the 3rd day after transplantation of the leukemia, followed 7 days later by injection of FCA, received a second course of treatment after 10 days.

In the course of the experiment the life span and survival rate of the animals were studied, and macroscopic and microscopic investigations were made (the mice were autopsied, and impressions of the bone marrow, liver, and spleen were studied). The numerical results were analyzed by statistical methods [4].

Laboratory of Experimental Therapy of Tumors, P. A. Gertsen, Moscow Oncologic Research Institute. (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. 72, No. 9, pp. 84-86, September, 1971. Original article submitted March 1, 1971.

TABLE 1. Effect of Treatment of Mice with Transplantable Leukemia-Hemocytoblastosis of Puyman's Strain La by Combined Administration of FCA and CP

| Scheme of treatment | Mean life span of mice | | Number of mice surviving until 180th day (in %) | P |
|---|---|---|---|---|
| | in days | in % | | |
| Control | 11.7 | 100 | 0 | – |
| FCA (3) | 10.9 | 93.2 | 0 | – |
| CP (3) | 37.3 | 318.8 | 0 | – |
| CP (3 and 20) | 61.5 | 525.6 | 13.9 | 0.02 |
| CP (3) + FCA (3) | 49.4 | 422.2 | 13.2 | 0.02 |
| CP (3) + FCA (10) | 93.9 | 802.5 | 42.9 | 0.001 |
| CP (3 and 20) + FCA (10 and 27) | 106.4 | 909.4 | 30.0 | 0.02 |

Note. Days after transplantation of leukemia are given in parentheses.

## EXPERIMENTAL RESULTS

As Table 1 shows, injection of CP into mice in a dose of 250 mg/kg on the 3rd day after transplantation of the leukemia led to an increase in the mean life span of the animals to 37.3 days (compared with a mean life span of the control mice of 11.7 days).

A second injection of the same dose of the compound 17 days after the first increased the mean life span of the mice to 61.5 days, and 13.9% of the mice in this group survived longer than 180 days.

The optimum single therapeutic dose of CP for the treatment of transplantable mouse hemocytoblastosis is 250 mg/kg body weight [1, 8]. This dose of the compound considerably depresses the leukemic process and considerably increases the life span of the mice (by 2-3 times) compared with untreated mice (control).

In the present experiments, although CP caused a lasting inhibition of the leukemia, it did not prevent recurrence of the disease, and this caused the death of most mice in these groups. In addition like most antitumor preparations, as well as inhibiting growth of the tumor, CP also depresses immunity. Investigations [6, 10] have shown that CP can give rise to the development of tolerance in animals to foreign antigen and can prolong the viability of an allogeneic skin graft.

The immunodepressive action of CP probably also facilitated to some degree the recurrence of leukemia in the present experiments.

Investigations [9, 11] have shown that the therapeutic effect of the medical treatment of leukemia can be significantly increased by stimulation of nonspecific immunity by means of various adjuvants.

In the present experiments, to stimulate the immune reactions of animals receiving CP, FCA was used. It had to be remembered that FCA, under certain conditions, can stimulate growth of virus tumors and leukemia [2, 5].

As Table 1 shows, FCA injected on the 3rd day after transplantation of the leukemia had no therapeutic effect. In the series in which FCA was injected simultaneously with CP on the 3rd day after transplantation of the leukemia, the mean life span of the animals exceeded by 12 days (103.4%) the mean life span of the mice receiving CP alone at the same times and in the same doses. In this group 13.2% of the animals survived more than 6 months without visible evidence of leukemia.

The maximum therapeutic effect was obtained when FCA was injected on the 7th day after injection of CP as a single dose. The number of animals surviving more than 180 days in this group without evidence of leukemia was 42.9% and the mean life span reached 93.9 days (an increase of 802.5% over the control).

Repetition of the same course of treatment after 10 days led to an increase in the mean life span of the animals by 909.4% over the control, and 30% of the mice survived. Treatment of the mice was accompanied by a decrease in the resistance of the animals to intercurrent infection, which was the cause of the low survival rate of the animals.

The results of postmortem examination of the animals from the various groups which died showed that the main cause of death during the 1st month of observation was the toxic action of CP.

A microscopic study of impressions of the spleen, liver, and bone marrow of the treated mice revealed solitary groups of leukemic cells against the background of severe aplasia of the hematopoietic tissue.

In half of the animals which died during the 2nd month of observation, definite signs of recurrence of the leukemia were observed. In the rest of the animals, pneumonia, enterocolitis, and other evidence of intercurrent infection were found. Intercurrent infection was the main cause of death of the mice in the later period up to the 180th day (i.e., until the time which can be taken as indicating virtual cure from the leukemia).

The results thus show that the use of CP in conjunction with FCA gives a marked therapeutic effect, under certain conditions, when transplantable leukemia-hemocytoblastosis of mice is used as the model.

The question of the mechanism of this effect requires special study by immunological methods. It may be supposed that administration of the adjuvant at a time when the antitumor action of the CP is at its maximum compensates for the immunodepressive action of the chemotherapeutic preparation and stimulates the development of the specific immune response.

## LITERATURE CITED


1. V. M. Bergol'ts and G. M. Sukhin, Probl. Gematol., No. 6, 36 (1968).
2. V. M. Bergol'ts, V. S. Ter-Grigorov, and O. Ya. Moskovkina, Vopr. Onkol., No. 9, 53 (1970).
3. A. A. Vorob'ev and N. N. Vasil'ev, Adjuvants (Nonspecific Structures of Immunogenesis) [in Russian], Moscow (1969).
4. I. A. Oivin, Pat. Fiziol., No. 4, 76 (1960).
5. V. S. Ter-Grigorov, I. S. Irlin, É. I. Tsysina, et al., Vpro. Onkol., No. 3, 53 (1969).
6. L. N. Fontalin, L. A. Pevnitskii, and V. V. Solov'ev, Byull. Éksperim. Biol. i Med., No. 11, 60 (1969).
7. N. Ya. Yurchenko and G. N. Platonova, Abstracts of Proceedings of a Plenum of the Department of Livestock Breeding of the All-Union Academy of Agricultural Sciences on the Problem of Leukemias [in Russian], Moscow (1966), p. 94.
8. S. Khadzhiev et al., Onkologiya (Sofia), 6, 16 (1969).
9. J. Amiel, J. Litwin, and M. Berardet, Rev. franc. Ét. clin. biol., 14, 909 (1969).
10. G. Mathé and J. Schwarzenberg, Rev. franc. Ét. clin. biol., 13, 695 (1968).
11. G. Mathé, Rev. franc. ét. clin. Biol., 13, 881 (1968).