COMPARATIVE QUANTITATIVE STUDY OF THE ANTILEUKEMIC ACTIVITY OF HEXAPHOSPHAMIDE, CERTAIN ANTILEUKEMIC PREPARATIONS, AND X-RAYS

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A study of the antileukemic action of hexaphosphamide on mice with hemocytoblastosis LA and on rats with erythromyelosis showed that the therapeutic index of this preparation is higher than that of thioTEPA. Hexaphosphamide is more effective than myleran, 6-mercaptopurine, methotrexate, colchamine, cyclophosphan, and x-rays for the treatment of erythromyelosis in rats.

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One of the urgent problems facing experimental therapy of the leukemias at the present time is the development of new and more specific antileukemic preparations of the ethylenimine series [4,6].

The object of the present investigation was to study the specificity and breadth of therapeutic action of one of the most active of the new ethylenimines (N_1N'-diethylene-N"-cyclohexyl triamidothiophosphate*), synthesized in the Laboratory directed by I. L. Knunyants at the Institute of Elemento-organic compounds of the Academy of Sciences of the USSR [1,2].

The preparation proved highly active when studied on strains of transplanted leukemias: it caused complete regression of erythromyelosis in rats and, compared with the control, significantly prolonged the life of mice with hemocytoblastosis LA (by 150%), L-1210 (by 62%), and less significantly in the case of mice with leukemia LEL (by 31%; it inhibited by 71-79% the development of lymphatic leukemias TsOLIPK No. 8 and TsOLIPK No. 30. In addition, the compound inhibited growth of Jensen's sarcoma by 99% and sarcoma 180 by 59% [5].

However, when new compounds are submitted to clinical trials, it has to be determined whether the preparation studied possesses advantages over compounds already known, closely related to them in structure.

Because of the similarity in principle between approaches to the chemotherapy of infections and of neoplastic processes [3], it would be beneficial to introduce quantitative methods into the chemotherapy of leukemias also [7].

EXPERIMENTAL METHOD

Hexaphosphamide is a white crystalline powder insoluble in water. It was introduced into the stomach as a suspension in 1% starch gel. The following strains of transplanted tumors were used in the investigation.

Erythromyelosis of rats. This was bred in the laboratory directed by Professor Svec (Czechoslovakia). It can be transplanted in 100% of cases into adult Wistar rats and also into inbred rats. After subcutaneous inoculation it grows into large tumors consisting of round cells resembling myeloblasts and solitary erythroblasts, reaching a weight of 77 g by the end of life and infiltrating the skin and subcutaneous

* By a decision of the Pharmacological Committee of the Ministry of Health of the USSR the preparation has been approved for clinical trials under the name "Hexaphosphamide."

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cellular tissue. The animals die on the average 16 ± 4.2 days after transplantation, with signs of spreading leukemic infiltration in the liver, spleen, lymph glands, kidneys and, less frequently, in the lungs. Neutrophilia, with large numbers of small segmented granulocytes of the pseudo-Pelger type are observed in the blood, and undifferentiated cells resembling myeloblasts and many erythroblasts are found. Numerous immature cells of the hyeloid series are observed in the bone marrow.

Acute Leukemia LA. This is transplanted in C57 black mice. It was bred by Puyman (Czechoslovakia). It is characterized by rapid generalization and by an increase in the number of leukocytes in the peripheral blood to 200,000 per mm^3. Massive leukemic infiltration takes place in the organs. I regard this leukemia as hemocytoblastosis. Antileukemic activity was assessed by the increase in the mean survival period over the control (in percent), using the formula [7]:

$$\frac{\text{Mean life span in experimental group} \times 100}{\text{Mean life span in control group}} - 100,$$

from the change in weight of the organs most severely affected by leukemic infiltration (the liver and spleen), and from morphological changes in the hemograms and myelograms and in impressions and histological sections of organs. The results were analyzed by statistical methods, the standard deviation and the criterion P being calculated.

The specificity of antileukemic action was assessed by plotting dose-effect curves on probit-logarithmic paper, doses being plotted in a range from ineffective to toxic. The preparations were compared by means of therapeutic indices, in which the ratio was calculated between the minimal tolerated dose (or the closely similar LD_{10}, more accurately reproducible graphically) and the dose causing a minimal therapeutic effect, which in the case of hemocytoblastosis LA was taken to be the dose increasing the survival period of the mice by 40%, and in the case of rats as the dose curing 90% of the animals with leukemia.

EXPERIMENTAL RESULTS

ThioTEPA increased the mean survival period of mice with hemocytoblastosis LA by a maximum of 25%, compared with an increase of 150% produced by hexaphosphamide in the optimal dose of 10 mg/kg (Table 1). The therapeutic index for hexaphosphamide was 2.6, while that for thioTEPA was less than 1.

Hexaphosphamide and thioTEPA, when injected in the maximal tolerated doses caused complete regression of erythromyelosis in rats if treatment began on the 5th day after transplantation. However, comparative quantitative assessment showed that the index for hexaphosphamide was 7.1, but that for thioTEPA was only 2.2 (Fig. 1).

TABLE 1. Action of Hexaphosphamide (I) and ThioTEPA (II) on Ascites Form of Hemocytoblastosis LA when Administered Daily

Preparation	Dose (in mg/kg)	Mean survival period (days)	Increase in length of survival (%)	Leukocyte count ($10^3/mm^3$)	Weight of spleen (in mg)
I*	30	6.7 (4.4–90)	7		65 (15–80)
I	20	10.2 (6.0–14.4)	41	2.6 (2.0–3.2)	107 (70–134)
I	10	18.0 (15.7–20.3)	150	5.0 (4.4–5.6)	189 (163–215)
I	8	11.2 (7.8–14.6)	55	10.1 (7.9–12.3)	670 (635–705)
I	5	7.6 (7.2–8.0)	5	13.1 (6.2–20.0)	680 (650–710)
I†	3	8.0 (3.5–12.5)	8	21.4 (12.9–29.9)	850 (820–880)
II†	8	7.6 (5.4–8.8)	5	1.9 (0.9–2.9)	55 (23–87)
II	6	9.0 (0.7–17.3)	25	2.6 (1.8–3.4)	70 (12–128)
II	4	8.2 (6.2–10.2)	14	7.7 (7.4–8.0)	399 (350–445)
II	2	7.8 (7.6–8.0)	8	12.2 (11.0–13.4)	580 (545–615)
Control		7.2 (6.7–7.7)		121 (115–127)	887 (557–1217)

*Given by mouth.
†Given subcutaneously.

TABLE 2. Action of Various Antileukemic Agents on Erythromyelosis of Rats when Administered Repeatedly from the 5th Day after Transplantation

Agent	Dose (in mg/kg)	Mode of administration	Number of doses given	Mean survival period (in days)	Percent of cures
Control	—	—	—	16.87 (15.1–18.5)	0
Myleran	2	Per os	10	19.5 (19.4–19.5)	0
6-Mercaptopurine	20	Intraperitoneally	10	17.1 (11.5–22.7)	0
Methotrexate. . .	0.2	»	6	15.5 (13.7–17.2)	0
Colchamine . . .	1	»	10	11.6 (9.0–14.2)	0
Cyclophosphamide	7	»	10	>90†	83.4
Leukeran		Per os	15	>90†	100
X-rays	75*	Whole-body Irradiation	10	19.1 (16.3–21.9)	0

*Dose rate in air 23 R/min.

†Surviving rats sacrificed with no signs of leukemia.

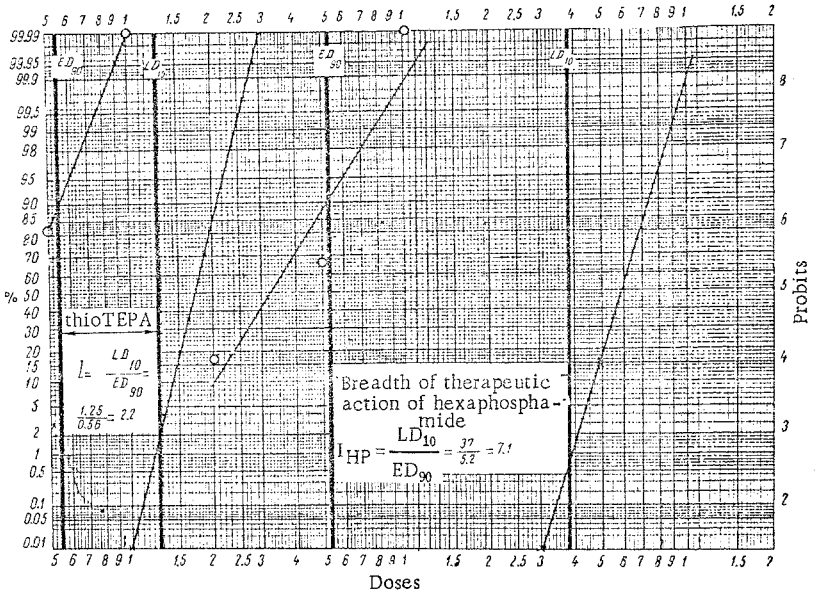

Fig. 1. Graph showing breadth of therapeutic action of hexaphosphamide and thioTEPA.

The results show that hexaphosphamide has much greater therapeutic activity than thioTEPA, although the latter was injected parenterally, ensuring much better conditions for absorption of the preparation.

The specificity of antileukemic action of hexaphosphamide also considerably exceeded that of any other agents (Table 2), with the exception of leukeran.

The data for the survival period given above are an integral index of antileukemic action, readily amenable to statistical analysis. The results of the corresponding hematologic and pathomorphological investigations, together with the biometric indices of the mean diameter of subcutaneous leukemic tumors were in full agreement with the data for the survival period and the percentage of animals cured from leukemia. These results, together with the absence of side effects of hexaphosphamide, other than inhibition of hematopoiesis, justify its recommendation for clinical study.

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