SUSTAINED DRUG RESISTANCE OF A TRANSPLANTABLE STRAIN OF RAT LEUKEMIA (ERYTHROMYELOSIS) TO PARAPHENACYL

E. I. Khomchenovskii

UDC 616-006.446-092.9-036.62+615.771.7-092.19

By transplantation of cells of a subcutaneous leukemic tumor (erythromyelosis of rats) recurring after administration of paraphenacyl (3.5 ml/kg, 10 times) a strain of the tumor resistant to paraphenacyl and other alkylating agents was obtained. The resistance persisted after repeated passages through healthy rats (over 1.5 years) without administration of the drug. The resistant strain is similar in its morphology and growth parameters to the sensitive variant.

* * *

Investigation of drug resistance of leukemias to active therapeutic agents, together with the search for new specific preparations, constitute some of the most important and urgent problems in experimental leukemia therapy.

Although resistance of leukemias to alkylating agents was discovered at the very beginning of their clinical application [4, 10], the experimental study of this problem began comparatively recently. Reports have been published of the development of resistance of a varying degree among experimental leukemias: Dunning IRC-741 of rats to embichin [8] and sarcolysin [12], leukemia 1210 of mice to cyclophosphamide [9], plasmacytoma of hamsters to TET and cyclophosphamide [11]. A number of investigations have been made of drug resistance of tumors to alkylating agents [1-3, 5, etc].

We have investigated the development of resistance to an active antileukemia preparation, paraphenacyl-N/p-di(2-dichloroethyl) aminophenacetyl/p-aminobenzoic acid-synthesized in the laboratory directed by Academician I. L. Knunyants of the Institute of Elemento-Organic Compounds, Academy of Sciences of the USSR. Paraphenacyl possesses high antitumor and antileukemic activity [6, 7].

EXPERIMENTAL METHOD

Experiments were carried out on 220 Wistar rats of both sexes weighing 200-300 g. Erythromyelosis of rats was obtained in Professor Švec's Laboratory (Czechoslovakia). As a result of passage of this strain for five years through rats from the "Stolbovaya" nursery the strain transplanted successfully in 100% of cases to cause death of all the animals after 16.8 (15.1-18.5) days ($P = 0.05$). The rats died with signs of generalized leukemia, with the appearance of pathological leukemic cells in the blood and bone marrow, extensive leukemic infiltration of the spleen, lymph glands, liver, and other internal organs, and with marked anemia. This strain of erythromyelosis is highly sensitive to compounds of the chloroethylamine and ethylnimine groups.

The normal and resistant variants of erythromyelosis were transplanted subcutaneously and bilaterally. A suspension of tumor cells and their complexes was obtained by mincing a subcutaneous leukemic tumor under sterile conditions and diluting it 1:3 in physiological saline. The control and experimental animals were weighed, their subcutaneous leukemic tumors were measured, the hemoglobin concentration and leukocyte and erythrocytes counts were determined and the blood picture studied; some animals were sacrificed for investigation of myelograms and of the histology of the organs.

Laboratory of Experimental Therapy of Leukemias, Central Institute of Hematology and Blood Transfusion, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR N. A. Fedorov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 65, No. 6, pp. 71-74, June, 1968. Original article submitted July 18, 1968.

TABLE 1. Dynamics of Acquired Resistance of Paraphenacyl in Strain of Erythromyelosis During 22 Generations Without Administration of the Drug

Generation of strain without treatment with paraphenacyl	Experimental conditions	Dose (in mg/kg)	Mean life span (in days; P=0.05)	Percent increase in life span	Percent cured of leukemia
0*	Paraphenacyl	3,5	>90	>450	83—100
0	Control	—	16,8 (15,1—18,5)	—	0
2	Paraphenacyl	7,0	18,0 (7,0—29,9)	10,0	0
2	"	3,5	17,7 (6,1—29,3)	10,0	0
2	Control	—	16,0 (12,2—19,8)	—	0
4	Paraphenacyl	7,0	18,3 (11,4—25,2)	3,0	0
4	"	3,5	20,3 (11,3—29,3)	3,0	0
4	Control	—	19,8 (16,7—22,9)	—	0
5	Paraphenacyl	7,0	17,1 (14,4—19,8)	2,0	0
5	"	3,5	16,5 (13,6—19,4)	0,0	0
6	"	7,0	18,5 (17,0—20,0)	4,0	0
6	Control	—	17,7 (15,4—20,0)	—	0
22	Paraphenacyl	3,5	18,8 (16,2—21,4)	18,8	0
22	Control	—	15,9 (14,8—17,0)	—	0

*Ordinary strain; rats sacrificed after 3 months without signs of leukemia. Paraphenacyl given by mouth in all experiments.

The intregal test of antileukemic activity readily amenable to mathematical analysis used in these experiments was the mean life span (MLS). The increase MLS in percent was determined with the formula [12]:

$$\left(\frac{\text{MLS of experimental control group}}{\text{MLS of control group}} \times 100\right) - 100.$$

The results of the measurements were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Administration of paraphenacyl in doses of 7 and 3.5 mg/kg 10 times or in a single dose of 60 mg/kg on the 5th day of transplantation of the erythromyelosis caused regression of subcutaneous leukemic tumors and of other manifestations of leukemia in 83-100% of rats. All the hematologic indices returned to normal and the animals recovered.

A strain of erythromyelosis resistant to paraphenacyl was obtained in November, 1964 as a result of transplantation of cells of a recurrent subcutaneous leukemic nodule after paraphenacyl treatment. When paraphenacyl was administered to 6 rats in a dose of 3.5 mg/kg for 10 days, 11 of the 12 subcutaneous tumors were completely absorbed. One tumor also diminished in size to 2 mm in diameter, but after stopping the drug, it grew again within two weeks to reach a diameter of 40 mm. This tumor was minced and transplanted into healthy rats which developed a typical picture of generalized leukemia, little different in its course from the original variant of erythromyelosis.

The dose of paraphenacyl causing regression of the ordinary strain of erythromyelosis was completely ineffective in the treatment of the strain derived from the recurrent cells. Practically complete refractoriness to paraphenacyl developed and the animals died with large tumors and signs of generalized leukemia at the same times as untreated animals (Table 1).

Resistance of the strain to paraphenacyl was maintained in subsequent generations by transplantation in the absence of the drug for more than 1.5 years. Observations on this strain are continuing.

The results of a study of the morphological peculiarities of the resistant strain of erythromyelosis showed very little difference between the cells of the normal and resistant variants. Cells of the resistant strain contain large and more clearly outlined nucleoli and their mitotic activity is slightly increased.

Changes in the bone marrow and the degree of specific leukemic infiltration of the internal organs were identical in both variants. The strain of erythromyelosis resistant to paraphenacyl also showed cross resistance to other chloroethylamine and ethyleneimine group agents.

The model of transplantable leukemia resistant to a particular group of compounds and maintaining its resistance at a stable level without further treatment with the preparation can be used to investigate a number of practical and theoretical problems.

In the strain of leukemia now obtained (with practically complete resistance to the drug) the growth parameters are similar to those of the ordinary strain sensitive to alkylating agents and its variant; this strain is a suitable model for studying the mechanism of action of the drug and the mechanisms of drug resistance; a comparative morphologic, genetic, and biochemical study of the normal and resistant variants of this leukemia is necessary.

The hypothetical mechanisms of onset of drug resistance in this particular case could be mutation followed by selection of resistant cells. This hypothesis is supported by the rapid onset of complete resistance and the sustained inheritance of this property without administration of the drug (for more than 1.5 years). Another possible mechanism could be selection of preexisting resistant cells, but this is contradicted by the possibility of complete cure, i.e., destruction of all leukemic cells after administration of optimal doses of paraphenacyl.

The resistance could be due to diminished permeability of the membranes of resistant cells to the drug, to the formation of metabolic by-passes for the formation of certain nonspecific metabolites, notably those containing sulfhydryl groups [13], which protect sensitive loci in the cells by competitively blocking alkylating groups capable of taking part in the reaction.

LITERATURE CITED

1. G. L. Zhdanov and L. F. Sharlikova, in: Problems in the Chemotherapy of Malignant Tumors [in Russian], Moscow (1960), p. 246.
2. G. L. Zhdanov and L. F. Sharlikova, Vopr. Onkol., No. 12, 26 (1961).
3. A. I. Kravchenko and A. A. Grushina, Vopr. Onkol., No. 5, 50 (1961).
4. L. F. Larionov, Treatment of Leukemia and Lymphogranulomatosis by Embichin [in Russian], Moscow (1951).
5. E. I. Komchenovskii and K. I. Karpavichus, Zh. Vsessoyuzn. Khim. Obshchest. im. D. I. Mendeleeva, 8, No. 4, 424 (1963).
6. E. I. Khomchenovskii, V. A. Odinokova, et al., Abstracts of Proceedings of the 41st Extended Plenum of the Scientific Council of the Central Institute of Hematology and Blood Transfusion [in Russian], Moscow (1964), p. 49.
7. L. F. Sharlikova, Vopr. Onkol., No. 6, 74 (1955).
8. W. B. Kessler, Proc. Am. Ass. Cancer Res., 3, 125 (1960).
9. M. Lane and S. T. Yancey, Nature, 188, 756 (1960).
10. C. P. Rhoads, J. Am. Med. Assn., 131, 656 (1946).
11. L. H. Simidt, Cancer Chemother. Rep., No. 16, 25 (1962).
12. H. E. Skipper and F. M. Schabel, Cancer Chemother. Rep., No. 22, 1 (1962).
13. G. P. Wheeler, Cancer Res., 23, 1346 (1963).