The Response of Transplanted Chloroleukemia in the Rat to Treatment with Antileukemic Drugs

By HARRY SHAY, CHARLES HARRIS AND MARGOT GRUENSTEIN

THE RECURRING QUESTION in cancer research regarding the application of results obtained with animal tumors to the human problem; the skepticism of such application often expressed by the investigators themselves and not infrequently the aversion shown by some experts engaged in clinical oncology to data obtained from experimental cancer in animals, bring into focus the need to establish as completely as possible the similarities as well as dissimilarities in anatomic characteristics and in chemotherapeutic response between any experimental tumor and the analogous tumor in man.

The valuable immediate and long-term effects of triethylene thiophosphoramide (Thio-TEPA) in the treatment of a transferable chronic myelogenous chloroleukemia in the rat has been adequately documented.[1] The method of maintaining this leukemia by animal transfer, its anatomic characteristics, its peripheral blood picture, its tissue metastasis, and the similarity of these to the findings in chronic myelogenous leukemia in man have been described.[1,2] The purpose of the present study, therefore, is not to critically evaluate over long periods the effects of various drugs on this leukemia, but rather to devise a short term screening program in order to determine whether its response would parallel that which is known to occur when these drugs are used in cases of chronic myelogenous leukemia in man.

METHOD

Test rats were inoculated intraperitoneally with leukemic cells within the first 7 days of life. Thirty days later, complete cell counts were made on samples of tail blood, and counts were repeated at weekly intervals thereafter until the diagnosis of leukemia could be established. Animals with leukemic cells in the peripheral blood and leukocyte counts of 20,000 cells/cu. mm. or more were chosen for the experiment. Because there is considerable variation in the incubation time between inoculation and the development of leukemia for individual animals, single experimental groups could not be treated simultaneously, but were gradually compiled as permitted by the number of leukemic animals available at any one time.

Rats harboring transferred myelogenous chloroleukemia and normal control animals of similar age were treated with triethylene thiophosphoramide (Thio-TEPA), triethylene phosphoramide (TEPA), ethyl carbamate (Urethane), 1,4-dimethanesulfonyloxybutane (Myleran) and 4-amino-N_{10} methyl pteroylglutamic acid (Amethopterin). Each drug was

From the Fels Research Institute, Temple University School of Medicine, Philadelphia 40, Penna.

This investigation was supported in part by research grant C-2206 from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

We are indebted to Dr. James Ruegsegger, Lederle Laboratories Division, American Cyanamid Company, for the supplies of triethylene thiophosphoramide, triethylene phosphoramide and 4-amino-N_{10} methyl pteroylglutamic acid.

We are indebted to Dr. Donald S. Searle, Burroughs Wellcome and Co., Inc., for the supplies of 1,4-dimethanesulfonyloxybutane.

Submitted June 20, 1955; accepted for publication September 29, 1955.

TABLE 1.—*Survival Data for Nonleukemic Control Rats*

	Dose Used	Solvent	14 Day Survival
Amethopterin	1 mg./Kg.	Saline	10/10
Myleran	10 mg./Kg.	Peanut Oil	6/10
TEPA	10 mg./Kg.	Peanut Oil	9/10
Thio-TEPA	10 mg./Kg.	Saline	10/10
Urethane	1 gm./Kg.	Saline	10/10

Drug administered daily for 6 days.

mixed with a suitable vehicle (table 1) and doses were administered by stomach tube for 6 consecutive days; therapy was then discontinued and 14 days after the initial treatment the surviving animals were sacrificed.

Complete cell counts were done on the peripheral blood of all experimental animals immediately prior to the first treatment, and on the blood of all survivors on the 7th and 14th day following the first treatment.

Except for Myleran, drug doses were maximized in each case by using the largest possible dose which failed to produce a significant mortality in nonleukemic rats. In the case of Myleran, pilot experiments indicated that doses of 5 mg./Kg. had no effect on the leukemia, whereas the best comparison between therapeutic efficiency and toxicity was achieved at a dose level of 10 mg./Kg. Twenty leukemic animals and 10 nonleukemic animals received identical treatment. Table 1 shows the dose and survival data for each drug in nonleukemic animals.

To compare the tissue changes produced by the different drugs in normal and leukemic rats with each other and with those produced by Thio-TEPA, which have been previously described,[1] the first three survivors of each group of treated leukemic animals and three treated nonleukemic animals were autopsied and their tissues as well as smears of femoral marrow were studied. We felt no need to repeat the histologic investigations on Thio-TEPA treated animals because of the previous extensive studies. Tissues were stained with hematoxylin and eosin, and McJunkin's benzidine technic for the demonstration of peroxidase. Marrow smears were stained with azure dyes and a benzidine technic adapted to the rat, and differential counts on 1000 cells were made for each sample.

RESULTS

In figure 1, the cumulative per cent of survivors is plotted as ordinate against the survival time (in days) of the experimental animal as abscissa. The results in this short term screening test show that, using survival time as the measure of effectiveness, Thio-TEPA, TEPA and Urethane were equally beneficial; that Amethopterin had no effect whatsoever, and that the action of Myleran was intermediate. The data in table 2 tend to support this interpretation and indicate further that whereas approximately only 17–30 per cent of survivors in the Thio-TEPA, TEPA and Urethane treated groups had leukemic cells circulating in the peripheral blood, such cells were found in 100% of the Myleran-treated animals. Figures 2 and 3 demonstrate the effect of the various drugs on the peripheral counts of nonleukemic controls and leukemic test animals.

Table 3 estimates roughly the degree of histologic evidence for leukemia in tissue and marrow for the 3 animals autopsied in each treatment group. Microscopic examination of the tissues of one of the three TEPA-treated animals failed to reveal any evidence of leukemia whereas the leukemic infiltrations found in the tissue of the other two appeared to be regressing as evidenced by small cells, shrunken nuclei and sparsely populated aggregates of leukemic cells.

The tissues of two Urethane-treated animals were similar in appearance to

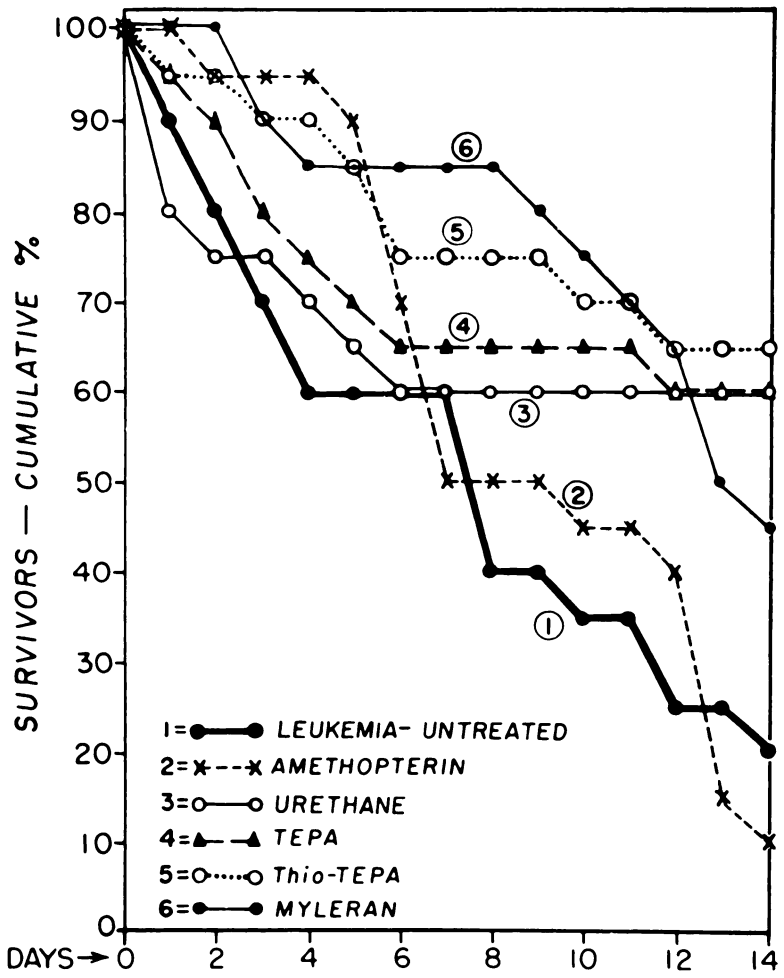

FIG. 1.—This compares the cumulative per cent survival during a 14-day period of six groups of leukemic rats treated as indicated. (100% = 20 rats.)

TABLE 2.—*14 Day Survival Data for Treated Leukemic Rats*

Animal Group	No. of Animals in each group that survived 14 days	No. of Surviving Animals that had leukemic peripheral blood counts
Thio-TEPA	13/20	4/13
TEPA	12/20	3/12
Urethane	12/20	2/12
Myleran	9/20	9/9
Amethopterin	2/20	2/2
Untreated	4/20	4/4

those of the latter animals but the third showed an advanced leukemic picture in both tissue sections and bone marrow smears.

Myleran-treated animals, despite their increased survival over the untreated controls, showed a pathologic picture which was indistinguishable from that

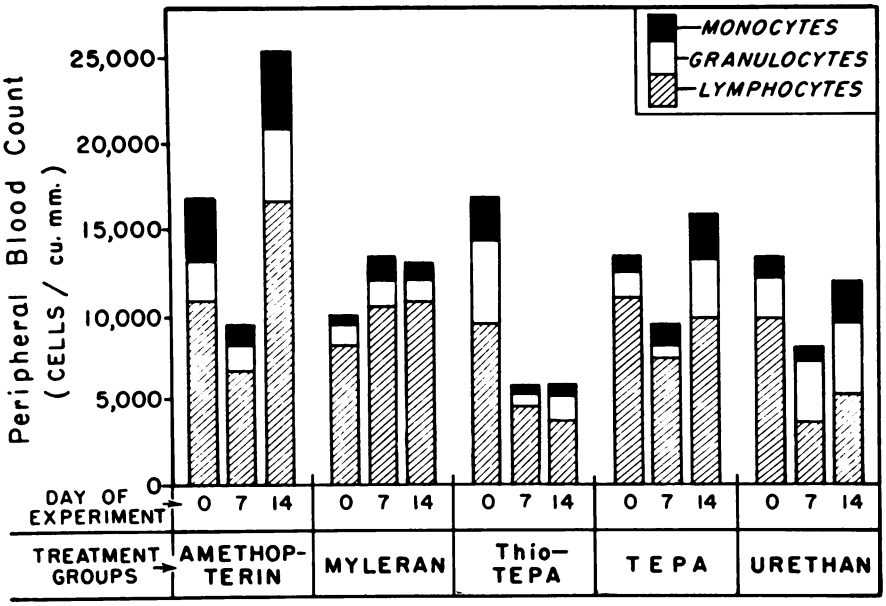

FIG. 2.—This compares the average peripheral blood counts of untreated nonleukemic rats to those of non-leukemic rats treated with the indicated drugs.

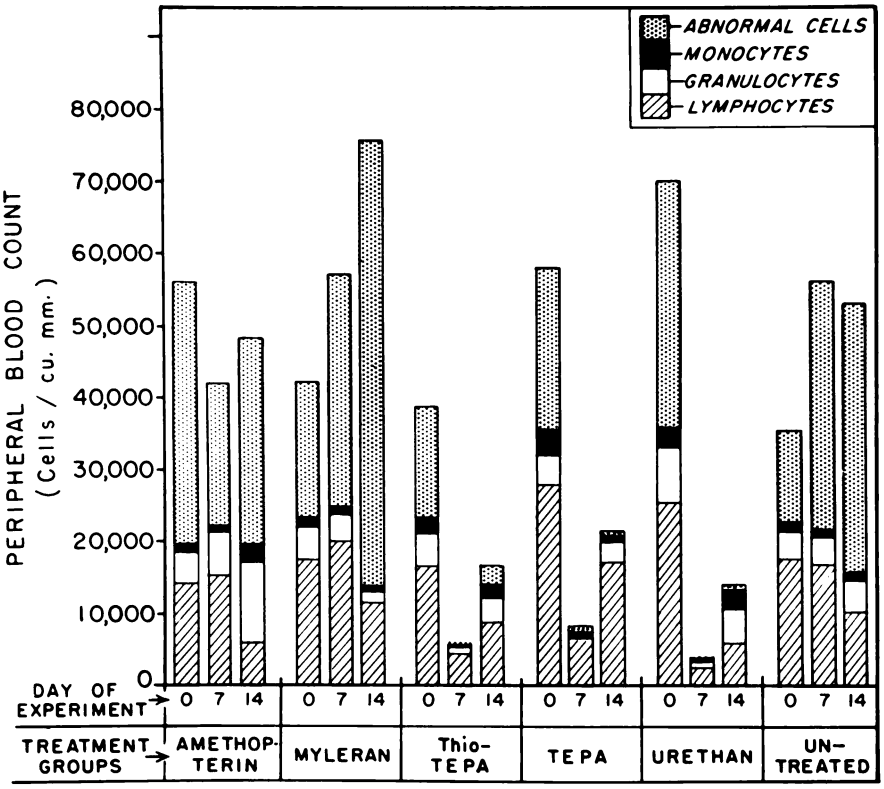

FIG. 3.—This compares the average peripheral blood counts of untreated leukemic rats to those of leukemic rats treated with the indicated drugs.

TABLE 3.—*Estimate of Degree of Leukemia Present in Tissues of 3 of the Animals that Survived 14 Days in Each Treatment Group*

Treatment Group	Animal	Presence of Leukemia (1-4+)		
		Tissues	Marrow	Peripheral Blood Counts
TEPA	H.256-54	+	−	−
	H.260-54	+	−	−
	H.271-54	−	−	−
Urethane	H.187-54	+	−	−
	H.189-54	++	−	−
	H.232-54	++++	+	+
Myleran	H.250-54	++++	++++	+
	H.249-54	++++	++++	+
	H.251-54	++++	++++	+
Amethopterin*	H.100-55	++++	++++	+
	H.101-55	++++	++++	+

* Only 2 of 20 animals survived for 14 days.

found in the Amethopterin treated rats and in the untreated leukemic controls.

The only significant pathologic change, other than those associated with the leukemic process, was the appearance of myeloid metaplasia, present to an abnormal degree in the livers and spleens of non-leukemic animals treated with Amethopterin. Slight myeloid metaplasia is a normal finding in the spleen of animals 8 to 10 weeks of age but the degree of this change can be increased when the animals are exposed to influences which depress the marrow.[3] In the liver the metaplasia was characterized by small collections of cells in the sinusoids, either small, dark and round nucleated red cells, or larger benzidine positive cells with reniform nuclei, ostensibly granulocytes. In the liver, these changes were superimposed upon an otherwise unchanged tissue, but in the spleen, they were associated with decrease in size of the malpighian corpuscles, disappearance of the normal mantle of light staining cells that normally surrounds this body in the rat, and a decrease in the number of erythrocytes in the red pulp. Instead, dense collections of cells derived from both the red and white cell series as well as numerous multinucleated giant cells so densely populated the red pulp that its entire function seemed given over to erythropoiesis and myelopoiesis. The spleens of leukemic animals treated with Amethopterin also exhibited myeloid metaplasia, the severity of which was obscured by the general overgrowth of peroxidase positive leukemic cells. No histologic difference was noted between the peroxidase positive cells found in foci of myeloid metaplasia and those which characterized the leukemia. Other than myeloid metaplasia, no specific pathologic changes in these animals could be attributed to the action of the drugs.

At 14 days after the first of six consecutive days of treatment, differential marrow counts of 1000 cells in the TEPA, Urethane and Myleran treated non-leukemic animals, and in 3 TEPA treated and in 2 of the 3 Urethane treated leukemic animals, were not different from those occurring in normal untreated animals of the same age group. In the Amethopterin group, however, 2 of 3 non-leukemic animals had increased peripheral white blood cell counts with associated granulocytosis of marrow, whereas the 3rd showed no change from the normal

in either marrow or peripheral blood counts. Peripheral blood counts of the entire Amethopterin nonleukemic group, after undergoing depression at 7 days, rebounded to relatively high levels by the end of the 14 day period (text-figure 2).

Discussion

In the short-term screening process employed in this study, Thio-TEPA, TEPA, and Urethane exhibited about the same degree of anti-leukemic activity. Myleran, however, appeared to have had an initial effect, which failed to persist beyond the first 8 days. At the end of the 14 day test period, although the number of surviving animals that were treated with Myleran was increased over the survivals of the untreated leukemic animals, the mortality rates of these two groups from the 8th to the 14th day ran parallel (fig. 1). Since comparison of these results with those obtained with the same drugs in myelogenous leukemia in man must be guarded because comparative data in a large relatively homogeneous group of cases in man are not available, nevertheless, the same drugs which are effective against this disease in man[2, 4, 5a, 6] exhibited antileukemic activity in the rat chloroleukemia, whereas Amethopterin, an active but impractical agent to use against myelogenous leukemia in man,[5b, 7, 8] also failed in the rat.

These effects indicate similarities of a qualitative biochemical nature between myelogenous leukemia in man and chloroleukemia in the rat beyond the morphologic resemblances already reported and serve to further qualify this experimental chloroleukemia as a tool for investigating the fundamental properties of myelogenous leukemia and for screening antileukemic agents since it provides an incomparable reservoir of leukemic cells.

Other than the obvious comparisons that the data yield, certain inferences relating to ancillary aspects of this problem are suggested from the histologic study of the tissues. Of prime importance among these is the fact that although ostensibly in complete remission, if judged by count of peripheral blood and even of marrow smears, two TEPA-treated animals and two Urethane-treated animals had residual microscopic foci of leukemic cells in their tissues probably capable of reinitiating the disease process. That relapse in man can occur after all hematologic evidence of disease is gone is documented,[2] and similar relapse in Thio-TEPA treated rats has been reported.[1] Therefore, to restate a question raised in a previous report, might it be wise with suitable chemotherapeutic agents, to push therapy beyond the point of hematologic remission in the hope of eradicating small viable foci of disease? At this stage there will be no clinical or laboratory indications of the disease, so that continued therapy would have to be guided solely by the toxic effects of the drug on the now normal peripheral blood and bone marrow pictures.

Table 4.—*Post-Treatment Values in Bone Marrow of Blasts Cells and Myelocytes Compared with These Values in Untreated Rats of Same Age Group*

	Normal	Nonleukemic Animals Treated With		Leukemic Animals Treated With	
		TEPA	Urethane	TEPA	Urethane
Blast Cells	0.7	0.4	0.8	0.8	0.8
Myelocytes	2.6	3.1	4.0	1.5	3.3

Heretofore, routine histologic methods have been sufficient to demonstrate the relatively gross effects of disease, but more refined technics such as those employed in the cytochemical demonstration of various enzymes, nucleic acids, amino acids and sugars, would have to be used were one to attempt to correlate a given drug with specific pathologic changes.

Table 4 compares the per cent of blast cells and myelocytes in the marrow of leukemic animals that responded to treatment with TEPA and Urethane as well as in non-leukemic animals treated with the same drugs to the per cent of these cells in the marrow of normal untreated rats of the same age. Although it is difficult and often impossible to distinguish between the leukemic cells and normal myelocytes by histologic methods, biochemical differences are indicated by the fact that TEPA and Urethane caused no depression of the normal immature elements either in nonleukemic or leukemic rats, when given in doses that had a devastating effect on leukemic cells. Whereas leukemic cells comprised 90% of pre-treatment leukemic marrows and no leukemic cells could be found in 5 of 6 post-treatment marrows (table 3), no concomitant reduction of normal blasts or myelocytes occurred (table 4).

Summary

Using a short-term screening program, we have demonstrated a parallelism in response between our myelogenous chloroleukemia in the rat and that of myelogenous leukemia in man when treated with a number of chemotherapeutic agents.

Summario in Interlingua

In un programma de selection a curte durantia nos ha demonstrate un parallelismo inter le responsa de chloroleucemia myelogene in rattos e le responsa de leucemia myelogene in humanos al tractamento con un numero de agentes chimotherapeutic.

REFERENCES

[1] Shay, H., Gruenstein, M. and Harris, C.: The effect of triethylene thiophosphoramide (Thio-TEPA) in the treatment of chronic myelocytic chloroleukemia in the rat. J. Nat. Cancer Inst. *15:* 3, 1954.

[2] ——, Zarafonetis, C., Smith, N., Woldow, I. and Sun, D. C. H.: Treatment of leukemia with triethylene thiophosphoramide (Thio-TEPA). Arch. Int. Med. *92:* 628, 1953.

[3] Lang, F. J.: Myeloid metaplasia. Handbook of Hematology, ed. by H. Downey, Paul B. Hoeber, Inc., 1938, p. 2120.

[4] Farber, S., Appleton, R., Downing, V., Heald, F., King, J. and Toch, R.: Clinical studies on the carcinolytic action of triethylene phosphoramide. Cancer *6:* 1, 1953.

[5] Wintrobe, M. M.: Clinical Hematology. 3rd edition, Philadelphia, Lea and Febiger, 1951, (a) pp. 868-870, (b) p. 874.

[6] Petrakis, N. L., Bierman, H. R., Kelly, K. H., White, L. P. and Shimkin, M. B.: The effect of 1,4-dimethanesulfonyloxybutane (GT-41 or Myleran) upon leukemia. Cancer *7:* 383, 1954.

[7] Burchenal, J. H., Karnofsky, D. A., Kingsley-Pillers, E. M., Southam, C. M., Myers, W. P. L., Escher, G. C., Craver, L. F., Dargeon, H. W. and Rhoads, C. P.: The effects of the folic acid antagonists and 2,6-diaminopurine on neoplastic disease. With special reference to acute leukemia. Cancer *4:* 549, 1951.

[8] Berman, L., Axelrod, A. R., Vonder Heide, E. C. and Sharp, E. A.: Use of a folic acid antagonist in chronic leukemia. Am. J. Clin. Path. *19:* 127, 1949.