Chemotherapy of Leukemia. V. Effects of 2,4,6-Triethylenimino-S-Triazine and Related Compounds on Transplanted Mouse Leukemia.* (18022)

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Certain ethylenimine and triazine derivatives have been of interest to the textile industry because of their chemical reactivity(1). The structural relationship of the ethylenimine groups to transformation products of the hydrolysis of nitrogen mustards and of the triazines to the pyrimidine moiety of pteroylglutamic acid suggested an evaluation of their chemotherapeutic activity in experimental cancer. Early in the study of this group one compound, 2,4.6-triethylenimino-*s-triazine* (SK 1133), demonstrated marked activity in prolonging the survival time of leukemic mice(2). Other investigators working independently have reported a retardation of tumor growth in mice by this compound(3). It has also been shown to inhibit various solid tumors in mice and rats(4,5) and to be active against mouse tumor explanted to the chorioallantoic membrane of the chick embryo (6). Since the activity of this compound might be attributed to either its triazine or ethylenimine ring structure, compounds possessing one or the other of these were tested in an attempt to demonstrate the active moiety of SK 1133. The results of these studies are herewith presented.

Methods. The technic for the evaluation of the chemotherapeutic activity of a given drug by means of its ability to prolong the survival time of mice with transmitted leukemia has been described previously(7,8).

Leukemia Ak4(9), a relatively acute strain, was used in these particular experiments. In a typical experiment, 240 mice of the inbred Akm stock were injected intraperitoneally with 0.1 cc of a saline suspension containing 1,000,000 cells. Forty-eight hours after injection these mice were divided into comparable groups of ten mice each (two sets of untreated controls, two sets of controls treated with a standard compound of known activity, 4-amino-N^{10}-methyl-pteroylglutamic acid, and twenty sets of mice treated with unknown compounds). Compounds were given intraperitoneally in maximum tolerated doses 3 times weekly for 10 doses. Water soluble compounds were dissolved in saline. Substances insoluble in water were usually suspended in 5% gum arabic in saline. The results of treatment with an unknown substance were compared with those obtained with the standard compound, 4-amino-N^{10}-methyl-pteroylglutamic acid, which has previously been shown to possess a high degree of chemotherapeutic activity against Ak4 leukemia(8,10). Maximum tolerated dosage was generally used throughout in an attempt

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TABLE I.
Effect of SK 1133 (A) on Transmitted Mouse Leukemia Ak 4.

Dose, mg/kg	Weight change		Untreated		Treated		
	Untreated*	Treated†	No. mice	Avg survival time (days)	No. mice	Avg survival time (days)	% increase
One gum arabic suspension used throughout entire course of injections.							
4	4.2	—0.2*	20	9.6	10	14.7	53
4	2.3	—3.2	19	13.3	6	26.8	101
4	0.8	2.4	20	11.7	9	25.2	115
4	2.5	—1.6*	19	11.7	9	14.8	26
4	1.8	—0.9	19	9.3	20	15.4	66
4	1.6	—1.9	20	9.6	10	15.5	62
2x1 4x9	—0.5	—1.4	20	11.4	9	26.1	129
2x1 4x9	1.7	—0.9	18	12.8	10	19.0	48
Gum arabic suspension prepared 24 hr before first inj. with same suspension used throughout entire course of inj.							
8	.7	—2.9	15	11.3	18	19.9	76
4	.7	0.3*	15	11.3	18	16.3	44
2	1.7	1.4*	18	11.9	10	12.9	8
Gum arabic suspensions prepared before each inj.							
1.5	1.5	—0.2	19	13.5	9	22.5	67
Saline suspension prepared before each inj.							
.75	4.7	—0.4*	18	10.2	10	17.0	67
.60	1.1	—2.9*	9	9.6	9	16.8	75
.55	2.1	0.6*	18	9.8	8	13.0	33
Filtered portion of saline suspension used throughout entire course of inj.							
1.0	1.9	—1.3	19	11.6	8	22.0	90
0.5	1.9	2.0*	19	11.6	10	12.8	10

* Wt change calculated as difference between initial wt and that one week later.
† Wt change calculated as difference between the initial wt and that 2 weeks later.

to procure the maximum effect, but in some experiments lower doses were employed. The mice were observed for the development of leukemia and autopsied at death. If gross evidence of leukemia was not conclusive, microscopic sections were taken.

Results. The results of the regular screening experiments with SK 1133 are presented in Table I. The drug was initially suspended in 5% gum arabic in saline. Later the possibility of a reaction between the gum arabic and SK 1133 was considered and tests were made of the activity of the compound suspended in saline. By this technic considerably higher toxicity was noted and smaller doses showed therapeutic effect. Later studies done by the Pharmacology Section(11) demonstrated that the insoluble fraction of the drug was only 2% by weight and that a filtrate of SK 1133 in saline retained the full toxicity of the original suspension. Although higher doses of the gum arabic suspensions had to be given, definite activity was shown by gum arabic suspensions, saline suspensions, or saline solutions when the maximum tolerated dose was employed. At half this dosage level very little effect was noted from the agent in any vehicle. At no tolerated dose was it as effective as 4-amino-N^{10}-methyl-PGA (Fig. 1).

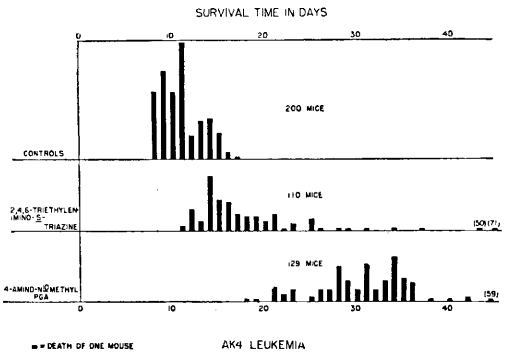

FIG. 1.

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TABLE II.
Effect of Certain Triazines and Ethylenimines on Transmitted Mouse Leukemia Ak 4.

Compound	Source of supply	Dose, mg/kg	Weight change, g		Untreated		Treated		% increase
			Untreated*	Treated*	No. mice	Avg survival (days)	No. mice	Avg survival (days)	
2,4-Diethylenimino-6-amino-*s*-triazine	A	.35§	2.1	—2.9†	18	9.8	9	18.6	90
		.35§	1.6	0.7	8	9.1	10	14.2	56
		.40§	1.6	—3.7†	20	9.6	7	25.1	161
		.40§	1.8	—2.2	9	8.9	9	16.5	85
2,4-Diethylenimino-6-phenyl-*s*-triazine	A	30	1.7	—0.3	18	11.5	8	13.3	16
		25§	2.2	1.6	17	9.0	9	10.3	14
		30x1§ 25x7§	1.9	0.5	20	10.7	10	13.6	27
		30‡	0.1	0	10	10.5	10	11.4	9
		35	—0.5	—0.6	19	9.4	10	12.2	30
2,4,6-Tris(dimethylamino)-*s*-triazine	A, B	125	3.9	0.2	17	13.6	9	18.6	36
		100	3.5	2.1	18	12.7	10	14.6	15
		100	2.2	—1.2	22	10.9	10	15.0	38
		50‖	2.0	2.1	10	10.6	10	12.4	17
		100§	2.2	1.1	17	9.0	10	9.6	7
Hexahydro-1,3,5-tris(methylacrylyl)-*s*-triazine	C	350¶	0.6	0.9	18	7.6	10	12.1	59
		500	1.6	1.5	8	9.1	8	11.4	25
		350	1.0	—2.4	18	10.7	8	14.0	31
Hexamethylene diethylenurea	A	10-15	0.7	—1.8†	15	11.3	7	21.3	97
		1§	—	0.5	10	7.2	10	16.1	124
		1.25§	0.1	—3.8†	10	10.9	7	16.0	47
Tolylene diethylenurea	A	1.5§	4.7	1.0	18	10.2	10	13.9	36
		2.0§	4.7	1.2	18	10.2	9	12.8	25
		3.0§	4.2	3.0	20	9.6	9	15.4	60

* Wt. change calculated as difference between initial wt. and 1 wk. later.
† 2 wks. later.
‡ Gum arabic suspension prepared before each injection.
§ Saline suspension prepared before each injection.
‖ One saline suspension used throughout entire course of inj.
¶ One propylene glycol sol. used throughout entire course of inj.

Table II lists the positive results obtained from experiments in which certain compounds containing a triazine or an ethylenimine component or both were tested for chemotherapeutic activity. Unless otherwise noted, one gum arabic suspension was used throughout the entire course of injections. On each compound tested the dosage was chosen on the basis of preliminary toxicity studies conducted in the Department of Pharmacology at Sloan-Kettering Institute under the direction of Dr. Frederick S. Philips. The letters listed beside each compound indicate the sources from which the materials were obtained for these experimental studies.†

The following chemically related compounds were also tested and they showed no significant effect against transmitted leukemia Ak4: Octadecylethylenurea, 2,4-Diamino-6-hydroxy-*s*-triazine, (supplied by A); 2,4-Diamino-6-n-propoxy-*s*-triazine, 2,4-Diamino-6-(2′-β-hydroxy-ethoxy-5′-arsonoanilino)-*s*-triazine, 2,4-Diamino-6-[4-*bis*(carboxy-methylenethio)arsenisoanilino]-*s*-triazine, 2,4-Diamino-6-(5′-arsono-2′-hydroxyanilino)-*s*-triazine, 2-Amino-4-morpholino-6-chloro-*s*-triazine, 2-Amino-4-cyclohexylamino-6-chloro-*s*-triazine, 2-Amino-4-N-propylamino-6-chloro-*s*-triazine, 2-Amino-4-methylamino-6-chloro-*s*-triazine, 2-Amino-4-ethylamino-6-chloro-*s*-triazine, 2-Amino-4-diallylamino-6-chloro-*s*-triazine, 2-Amino-4-(β-hydroxyethyl-ethylamino)-6-chloro-*s*-triazine, 2-Amino-4-(N-β-hydroxyethylanilino)-6-chloro-*s*-triazine, 2,4-Bis-methallylamino-6-chloro-*s*-triazine, 2,4-Bis(dimethallylamino)-6-chloro-*s*-triazine, 2,4-Bis(β-hydroxypropylamino)-6-(4′-arsonoanilino)-*s*-triazine, 2,4-Diallylamino-6-chloro-*s*-triazine, 2-Methallylamino-4-dimethallylamino-6-chloro-*s*-triazine, 2-Dimethylamino-4,6-dichloro-*s*-triazine, 2-Ethylamino-4-diethylamino-6-chloro-*s*-triazine, 2,4-Dipiperidino-6-chloro-*s*-triazine, (supplied by B); Hexahydro-1,3,5-triacrylyl-*s*-triazine, (supplied by C); 2,4-Bis(hydroxymethylamino)-6-(α-hydroxymethyl)-benzyl-*s*-triazine, (supplied by D); 2,4-Diamino-6-benzyl-*s*-triazine, 2-Amino-4,6-diacetamido-*s*-triazine, (supplied by E); 2,4-Diamino-*s*-triazine, 2,4-Diamino-6(β-dimethylamino-ethoxy)-*s*-triazine, (supplied by F); 2,4-Bis(aminomethylamino)-6-bis(aminomethyl)amino-*s*-triazine, (supplied by E and H); 2,4,6-Triamino-*s*-triazine, (supplied by A and F); 2,4,6-Trihydroxy-*s*-triazine, (supplied by B, C, and G); 2,4,6-Trimethylolamino-*s*-triazine, (supplied by A and I); 2,4-Diamino-6-chloro-*s*-triazine, (supplied by F and G); 2,4-Diamino-6-mercapto-*s*-triazine, (supplied by D, E, and B); 2,4-Diamino-6-(4′arsonoanilino)-*s*-triazine, (supplied by B and G).

Discussion. The results of these experiments demonstrate that in this series the compounds showing the ability to prolong significantly the survival time of mice with transplanted leukemia Ak4 all possess at least 2 ethylenimine rings. The possession of a triazine or melamine moiety by the active agent does not seem to be essential since one compound, hexamethylene diethylenurea, tested in this series could not be classified as containing either of these groups but appeared to possess definite activity by virtue of the ethylenimine rings. In these studies none of the compounds possessing a single ethylenimine ring prolonged significantly the survival time of leukemic mice. This is analogous to the results obtained against transmitted mouse leukemia with the nitrogen mustards (7) where no agent had chemotherapeutic effect against leukemia unless it possessed at least 2 beta halogenated alkyl groups. In studies on the effect of this type of compound on the leukocyte count of the leukemic mouse(12), however, ethylenimine and 2,4-dimethoxy-6-ethylenimino-*s*-triazine, as well

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A—Calco Chemical Co.

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C—Goodrich Chemical Co.

D—Abbott Laboratories.

E—Monsanto Chemical Co.

F—G. D. Searle and Co.

G—Dr. Ernst. A. H. Friedheim.

H—Chemical-Biological Coordination Center of the National Research Council.

I—Imperial Chemical Industries, Ltd.

12. Burchenal, J. H., Biedler, J. L., Meigs, G. M., and Nutting, J., unpublished data.

as the compounds mentioned here as showing therapeutic activity, caused a definite fall in the leukemic leukocytes in the peripheral blood. Clinical trials with SK 1133 (triethylene melamine) have shown it to possess chemotherapeutic activity similar to that of methyl-bis(β-chloroethyl)amine against Hodgkin's disease, lymphosarcoma, and the chronic leukemias(13). It would seem valid, therefore, to attribute the activity as chemotherapeutic agents, of the particular series of compounds discussed in this report, to the nitrogen mustard-like ethylenimine ring rather than to the triazine or melamine structure.

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Summary. 1. 42 compounds, of which 39 could be classed as triazines and 3 as ethylenimine ring compounds were screened for chemotherapeutic activity against Ak4 mouse leukemia. 2. 2,4,6-triethylenimino-*s*-triazine, 2, 4 - diethylenimino - 6 - amino - *s* - triazine, and hexamethylene diethylenurea significantly prolonged the survival time of mice injected with transplanted leukemia Ak4. 3. It would appear that the chemotherapeutic activity of the above compounds can be attributed to the ethylenimine structure. 4. Four other compounds containing either a triazine or an ethylenimine moiety showed a slight chemotherapeutic effect.

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Inactivation of Estradiol by the Hepatic Tissues of Mice.* (18023)

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Because of the role that estrogens play in experimental carcinogenesis and in many clinical conditions, considerable significance is attached to any factor which would increase or decrease the titer of circulating estrogens. The liver destroys estrogen in a majority of laboratory animals(1-4). Marked damage to the liver in these animals caused a rise in titer of the circulating estrogen(5,6). Factors which will decrease the liver's ability to inactivate estrogen include carbon tetrachloride toxicity(5), virus hepatitis(7), partial hepatectomy(6), and nutritional deficiency(8,9). An important aspect of this problem is: what is the effect of large quantities of estrogen in the blood upon the liver's ability to inactivate estrogen?

The effect of a prolonged castrate condition and of prolonged hyperestrogenemia upon the ability of the mouse's liver to inactivate estrogen was studied.

Methods. Animals used were first generation hybrid mice, designated CC_1, CC_2, AC_1, AC_2, AC_3, and AC_4. A few inbred mice of the C_{57}, NHO and CHI strains were used. The mice were divided into 3 groups, one group was untreated, mice of the second group were castrated, and mice of the third group were castrated and then 2 mg pellets of estrone were implanted subcutaneously. Six weeks, 8

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