Studies on the Effects of a Guanine Analog on Acute Lymphoid Leukemias of Mice*

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Roblin and associates (9) in 1945 synthesized the guanine analog, 5-amino-7-hydroxy-1*H*-*v*-triazolo (*d*) pyrimidine which has been referred to by Kidder *et al.* (6) in a subsequent study as guanazolo. It has been found that in bacteria (9) and in *Tetrahymena geleii* (7) competitive inhibition of growth results following the use of this substituted pyrimidine. Release or reversal of growth inhibition was accomplished by guanine in both organisms.

TABLE 1

EFFECT ON BODY WEIGHT, ADRENAL WEIGHT, AND THYMUS WEIGHT IN CAF_1 MICE INJECTED WITH GUANAZOLO*

Dosage (μg/gm) daily	Dosage (μg/gm) total	Per cent change body weight (range mean weights)	Adrenal weight† (per cent body weight)	Thymus weight (per cent body weight)
0	0	+1.1 (19.6–19.8)	.035	0.30
25	250	−4.3 (18.2–17.4)	.034	0.36
50	500	+1.5 (19.1–19.4)	.035	0.34
75	750	−1.6 (18.5–18.2)	.043	0.26
112.5	1125	+5.8 (17.0–18.0)	.041	0.13

* Nonleukemic CAF_1 females injected daily with guanazolo for 10 days. Control mice injected similarly with 0.01 N NaOH. Five mice in each group.

† Paired adrenal weights. Adrenal and thymus weights measured on torsion balance.

Retardation of the growth of a transplantable mammary adenocarcinoma and of spontaneous mammary cancers in mice was reported, following injections of guanazolo suspended in distilled H_2O. Data on the inhibitory effect of this compound on a transplantable acute lymphoid leukemia were not conclusive. Attempts at reversal of the guanazolo inhibition in mice by the use of guanine were unsuccessful (5).

Burchenal *et al.* (1) reported no inhibitory effect of three triazoles, including guanazolo, on survival time of mice bearing a transplantable lymphoid leukemia AK-4, although a purine derivative, 2,6 diaminopurine, which has been found to be an antagonist of pteroylglutamic acid and of adenine (4), caused a definite increase in survival time of mice bearing the same transplantable leukemia (2).

The effect of guanazolo[1] on two transplantable acute lymphoid leukemias in mice, leukemia L 1210 (strain dba) and Lymphoma 2 (strain A), and of a lymphosarcoma, Lymphoma 1 (strain A), has been investigated. Description of these lymphomas has been given previously (8). Guanazolo was given at the following dosage levels to leukemic mice: 12.5, 25, 37.5, 50, 75, and 112.5 μg/gm body weight. The compound in solution in 0.01 N NaOH was injected subcutaneously daily, the total number of injections depending upon the type of leukemia used and upon the condition of the animals. A 0.1 per cent solution was used for the 12.5, 25, and 50 μg/gm dosage levels, and a 0.133 per cent solution (100 mg/75 cc) was used for the 37.5, 75, and 112.5 μg/gm dosage levels. Fresh solutions were prepared each week.

In a preliminary experiment we have observed the effects of different dosage levels of guanazolo in normal, nonleukemic CAF_1 mice (♀ C × ♂ A cross). No demonstrable effect on body weight or on the blood picture (hemoglobin, total and differential leukocyte counts) was observed after ten daily subcutaneous injections at dosage levels 25–112.5 μg/gm body weight. At the higher dosage levels, 75 and 112.5 μg, definite adrenal hyperplasia and thymus involution were evident, however (Table 1).

Definite, regular, and reproducible increase in survival time was found in both acute lymphoid leukemias following administration of guanazolo at different dosage levels (Tables 2 and 3). A total of 156 dba mice in twelve different experiments

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TABLE 2

Effect of Guanazolo on Acute Lymphoid Leukemia L 1210*

Experiment	Compound	Number of mice†	Dosage (μg/gm) Daily	Dosage (μg/gm) Total	Survival time mean range (days)	Increase in survival time	Mean‡ wt. change (gm.)
	Guanazolo	4	50	400	13.7 (9–17)	52 per cent	+0.7
1	"	8	25	200	15.1 (12–17)	68 per cent	+1.8
	Controls§	11			9.0 (9. 0)		+1.8
	Guanazolo	6	50	550	13.7 (12–15)	51 per cent	+0.7
2	"	8	75	825	13.9 (13–16)	53 per cent	−0.3
	Controls	7			9.1 (9–10)		+2.2
	Guanazolo	5	112.5	1,125	10.8 (9–14)	3 per cent	+0.2
3	"	5	50	500	12.2 (8–15)	16 per cent	+1.2
	Controls	5			10.5 (9–12)		+2.2
	Guanazolo	8	25	225	12.0 (9–15)	30 per cent	+1.9
	"	8	50	450	13.0 (11–14)	41 per cent	+0.8
4a	"	8	75	675	13.4 (9–18)	45 per cent	+0.3
	Controls	5			9.2 (8–12)		+2.6
	Guanine HCl	5	375	3,750	9.6 (8–11)		+0.9
	Guanazolo‖	5	25	250	14.4 (14–15)	22 per cent	+0.5
4b	Controls	5			11.8 (10–14)		+1.1
	Guanazolo	5	25	300	14.0 (10–17)	52 per cent	+0.4
5	Controls	5			9.2 (9–10)		+1.8
	Guanazolo+		25	300			
	Guanine HCl	8	375	4,500	10.2 (9–11)		−0.5
	Guanazolo	8	25	150	10.0 (8–12)	25 per cent	+2.0
	"	8	37.5	225	10.9 (9–13)	36 per cent	−0.5
6	Controls	8			8.0 (7– 9)		+2.4
	Guanazolo+		25	150			
	Guanine HCl	8	375	2,250	10.0 (9–11)		+0.4
	"		37.5	225			
	"	8	375	2,250	9.1 (7–10)		−1.0
	Guanazolo	9	50	450	11.3 (11–12)	24 per cent	+1.0
	Controls	9			9.1 (8–12)		−0.5
	Guanazolo+		50	300			
7	Guanine HCl#	8	375	2,250	9.4 (7–12)		−1.4
	"		25	150			
		8	375	2,250	8.0 (7– 9)		−1.4
	"		25	150			
		8	375	2,250	9.1 (8–12)		−1.0
	Guanazolo	10	50	600	12.6 (11–15)	29 per cent	+0.2
8	"	10	75	765	12.6 (10–15)	29 per cent	−0.2
	Controls§	10			9.8 (9–11)		+0.4
	Guanazolo	12	12.5	100	10.0 (9–11)	11 per cent	−0.4
9	"	13	112.5	900	11.6 (8–14)	29 per cent	−2.8
	Controls	8			9.0 (9.0)		+0.2
	Guanazolo	10	50	400	10.5 (10–11)	21 per cent	+1.1
	"	9	75	600	11.0 (8–13)	27 per cent	−1.3
10	Controls	10			8.7 (8–11)		+1.7
	Guanazolo+		50	400			
	Guanine HCl#	10	375	3,000	9.4 (7–10)		−1.9
	Guanazolo	8	37.5	300	11.7 (10–13)	29 per cent	0.0
	"	8**	50	250	11.1 (9–12)	22 per cent	+0.5
11	Controls	8			9.1 (8–10)		+0.3
	Guanazolo+		37.5	300			
	Guanine HCl#	9	375	3,000	10.0 (9–11)		−2.8
	Guanazolo	5	25	300	13.8 (13–15)	50 per cent	+0.9
12	"	6	50	400	15.2 (11–16)	65 per cent	+0.4
	"	5	75	525	15.4 (11–15)	67 per cent	−0.3
	Controls§	5			9.2 (8–12)		+2.6

* All inoculations of leukemic cells, except where noted, done intraperitoneally with leukemic cell brei. Approximately 800,000 cells inoculated in 0.1 cc. volume.

† Strain dba mice, 15–20 gm. used throughout. Since a sex difference in response was not noted, sexes are combined.

‡ Weights obtained 8 days after beginning of experiment.

§ Controls injected with 0.01 N NaOH.

‖ Inoculations of leukemic cells done subcutaneously.

Guanine HCl suspended in 5 per cent gum arabic in saline. In all other experiments guanine suspended in peanut oil. Controls in experiments 10 and 11 received 5 per cent gum arabic in saline.

** Guanazolo administered 96 hours after inoculation of leukemic cells.

TABLE 3

Effect of Guanazolo on Acute Lymphoid Leukemia, Lymphoma 2*

Experiment	Compound	Number of mice	Dosage Daily	μg/gm Total	Survival time mean range (days)	Increase in survival time	Mean† weight change (gm.)
	Guanazolo	7‡	50	600	15.4 (14–17)	37 per cent	+0.6
1	"	7	75	750	16.3 (14–20)	44 per cent	−0.3
	Controls	7§			11.3 (11–12)		+1.8
	Guanazolo	8	25	250	13.9 (11–21)	17 per cent	+0.9
2	"	8	50	500	14.5 (12–19)	22 per cent	−0.7
	Controls	9§			11.9 (11–15)		+1.4
	Guanazolo	8	37.5	375	13.9 (11–16)	42 per cent	+0.5
3	"	8	75	750	15.5 (13–19)	58 per cent	−0.4
	Controls	6			9.8 (9–10)		+2.2
	Guanazolo	10	25	250	12.9 (12–15)	11.2 per cent	−1.0
4	"	11	50	500	14.2 (12–20)	23 per cent	−0.3
	"	11	75	750	14.0 (13–16)	21 per cent	−1.3
	Controls	8			11.6 (11–12)		+0.5
	Guanazolo	8	25	225	11.9 (11–13)	17 per cent	+1.0
5	"	8	50	400	12.4 (11–14)	22 per cent	−0.7
	Controls	15			10.2 (9–11)		+0.3

* All inoculations with leukemic cell brei done intraperitoneally. Approximately 800,000 leukemic cells inoculated in 0.1.-cc volume.

† Weights obtained 10 days after beginning of experiments.

‡ CAF_1 mice 20–25 gm. body weight of mixed sexes used throughout.

§ Controls injected with 0.01 N NaOH.

TABLE 4

Summary of Results of Tables 2 and 3

Compounds used	Dosage (daily) (μg/gm)	Number of mice	Mean survival time—days	Increase in survival time
		Leukemia L-1210		
Guanazolo	25	34	12.9 (10.0–15.1)	41.7 per cent
"	37.5	16	11.3 (10.9–11.7)	24.2 per cent
"	50	66	12.4 (10.5–15.2)	36.3 per cent
"	75	40	13.0 (11.0–15.4)	42.9 per cent
Guanazolo+ Guanine HCl	25, 37.5, and 50 375	67	9.4 (8.0–10.2)	
Controls		91	9.1 (8.0–10.5)	
4-amino-N^{10}-methyl-PGA*	3	41	21.0 (14.4–26.6)	130.8 per cent
		Lymphoma 2		
Guanazolo	25	26	12.9 (11.9–13.9)	18.3 per cent
"	37.5	8	13.9 (11.0–16.0)	27.6 per cent
"	50	34	14.1 (12.4–15.4)	29.3 per cent
"	75	26	15.1 (14.0–16.3)	38.5 per cent
Controls		45	10.9 (9.8–11.9)	
4-amino-N^{10}-methyl-PGA*	3	16	14.4 (11.0–17.0)	32.1 per cent

* Injections done intraperitoneally every other day.

TABLE 5

Comparative Blood Studies of Strain DBA Mice Inoculated with Leukemia L 1210*

Compound	Number of mice	Hb. gm. per cent	Reticulocytes	wbc	Granulocytes	Agranulocytes	Blasts
Guanazolo (37.5 μg/gm)	5	11.7 (11.5–12.0)	0.1 per cent	42,700 (28,000– 52,000)	49.4	45.3	5.3
Guanazolo+ Guanine HCl (37.5 μg/gm+375 μg/gm)†	5	10.7 (9.5–12.0)	0.4 per cent	60,700 (38,250– 89,750)	32.7	50.7	16.6
Controls	5	12.3 (11.0–13.0)	2.0 per cent	119,600 (67,000–197,000)	39.0	35.7	25.3
4-Amino-N^{10}-methyl-PGA	3	13.3 (12.7–13.5)		11,800 (10,500– 13,000)	35	63	2.0

* Blood studies done 8 days after inoculation of leukemic cells.

† Guanine HCl in 5 per cent gum arabic in saline given orally daily.

and 94 CAF_1 mice in five different experiments were used for studies on survival time. Table 4 summarizes the results of the individual experiments and compares the effects obtained, using guanazolo, with the effect of 4-amino-N^{10}-methyl folic acid (amethopterin), the most effective of the folic acid antagonists in retarding leukemic growth in our experience. The antifolic compound produced a 130.8 per cent increase in survival time in dba mice inoculated with leukemia L 1210, compared with a 37.9 per cent increase with guanazolo. Guanazolo was as effective as 4-amino-N^{10}-methyl folic acid in increasing survival time in CAF_1 mice inoculated with Lymphoma 2.

Guanazolo was effective over a wider dosage range in dba mice bearing leukemia L 1210 than in strain A mice bearing Lymphoma 2. Increase in survival time was as great at the dosage level, 25 μg/gm body weight, as at the higher level, 75 μg/gm body weight, in strain dba mice with leukemia L 1210, whereas in strain A mice with Lymphoma 2 it is indicated that guanazolo becomes increasingly effective with an increase in dosage. A definite toxicity was apparent in dba leukemic mice at dosage levels of 112.5 μg/gm body weight. Death of a small percentage of mice at this dosage level occurred before death of control leukemic mice, although typical changes characteristic of the folic acid deficiency syndrome, viz., hypoplasia of bone marrow, edema of the intestinal tract with associated desquamation, and diarrhea were not observed. Toxic symptoms were also apparent at this dosage level in normal, nonleukemic CAF_1 mice (Table 1). The discrepancy between these results and those of Kidder *et al.* (6), who stated that they observed no toxic effects of the guanazolo, may be explained by the difference in dosage levels, since their material was used in suspension and ours in solution.

Table 5 shows the effect on the blood picture in dba leukemic mice injected with guanazolo. Control animals developed a florid leukemia with a severe leukocytosis, and 25 per cent blast forms were found in the peripheral blood. Although a moderately high leukocyte count was found in guanazolo-treated mice at the 8-day level, relatively few blast cells were present. The inhibitory effect of the folic acid antagonist, 4-amino-N^{10}-methyl folic acid, on leukemic dba mice was found to be more pronounced 8 days after inoculation of leukemic cells, and the blood picture closely resembled that of the normal nonleukemic mouse.

Definite arrest of the leukemic process was evident in histologic sections of the spleen, liver, and lymph nodes. Moderate infiltration of leukemic cells into these organs was found, in contrast to the extensive infiltration in control mice. The marked reduction or complete absence of leukemic infiltration seen in leukemic mice given parenteral 4-amino folic acid (8) was not found in any of the mice examined in this study.

Marked reduction in the rate of growth of leukemic cells of acute lymphoid leukemia L 1210 inoculated subcutaneously was observed 8 days after inoculation (6 days after the beginning of parenteral administration of 50 μg/gm guanazolo). At this time, the local mass of lymphoma tissue weighed 566.2 mg. (five mice), while the mean weight of lymphoma tissue from guanazolo-treated mice was 213.4 mg. (five mice). It is pertinent to note that guanazolo at the same dosage level was effective in adrenalectomized mice. The mean weight of the subcutaneous lymphoma mass of adrenalectomized mice was 168.6 mg. (five mice).

No evidence of necrotic changes have been observed in lymphoma tissue grown in the subcutaneous regions or in leukemic cells infiltrating other organs.

No effect on survival time of CAF_1 mice bearing a lymphosarcoma, Lymphoma 1, or on the rate of growth of lymphosarcoma tissue was noted following daily subcutaneous injections of guanazolo at the dosage level 50 μg/gm. The survival time for controls was 39.7 days and for guanazolo-injected mice (total dose 463 μg/gm body weight) was 32.3 days (25 mice in each group).

In an attempt to arrive at some understanding of the mode of action of guanazolo in the inhibition of the growth of leukemic cells, release of inhibition was sought by the use of guanine HCl. Subcutaneous injections of guanine HCl in suspension in peanut oil or in 5 per cent gum arabic in saline were commenced 24 hours after inoculation of leukemic cells (24 hours prior to parenteral guanazolo treatment) and continued daily 4 hours before injection of the guanine analog. The dose used was 10–15 times by weight that of the analog (375 μg/gm body weight given in 0.2 cc. daily). This was reported to be the ratio of metabolite to inhibitor required to release completely the inhibition of guanazolo in *Tetrahymena* (7).

It can be seen, by reference to the individual experiments in Table 2 or in the summary in Table 4, that, judged on survival time, a release of the antileukemic effect of guanazolo is obtained. The survival time of dba mice bearing acute lymphoid leukemia L 1210 is essentially that of the untreated leukemic controls (9.4 days compared with 9.1 days). The gross and microscopic appearance of the leukemic process in these animals was similar to that seen in the leukemic control mice. Release of inhibition, as judged by the peripheral blood

picture, however, was only partial (see Table 5), although the leukocyte count was definitely higher, and the number of blast cells found approached that in the dba leukemic mouse. It should be noted that slight loss of weight was encountered in each experiment where metabolite and inhibitor both were given, probably because of the appearance of sterile abscesses in some of the mice at the site of inoculation of guanine HCl. In experiments now in progress, however, apparent release of inhibition is obtained without loss of weight, following oral administration of guanine HCl, and it is indicated that lower ratios of metabolite to inhibitor are effective.

Release of the anti-leukemic effect has not yet been accomplished by the use of pteroyltriglutamic acid or adenine sulfate.

It would seem from the available data that a relative deficiency of guanine is established by parenteral administration of the guanine analog, guanazolo, and that leukemic cells require a more abundant supply of guanine than do normal cells of the lymphoid series. A parallel situation seems to occur in leukemic mice given the folic acid antagonists, 4-amino folic acid and 4-amino-N^{10}-methyl folic acid, where the leukemic process may be controlled by an apparent slight deficiency of folic acid without seriously impairing the processes for normal cell life. The anti-leukemic effects of both of these folic acid antagonists have been blocked by folic acid or its derivatives (8, 3).

In general, it has been found that similar anti-leukemic effects have been shown by the antifolic compounds and by 2,6 diaminopurine and the guanine analog guanazolo. Whether or not the same locus of action is characteristic of all will be revealed by more complete reversal and hematologic studies.

SUMMARY

1. A definite, regular, and reproducible inhibition of two transplantable acute lymphoid leukemias in mice resulted from parenteral administration of the guanine analog, guanazolo.

2. No effect of this compound on a transplantable lymphosarcoma was observed.

3. Apparent release of inhibition by the use of guanine HCl is recorded.

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