Antitumor Activity of 5-Bis(2-Chloroethyl) Amino Uracil Against Sarcoma 180 and Ehrlich Carcinoma. (24439)

J. S. Evans and G. D. Mengel*
Research Division, The Upjohn Co., Kalamazoo, Mich.

Incorporation of chloroethylamine groups into compounds of biological activity may lead to compounds which have selective action on certain types of neoplasms. l-Phenylalanine nitrogen mustard (CB-3025)(1), quinacrine nitrogen mustard(2), and Degranol (BCM)(3) may be considered as examples of compounds of this type. Lyttle and Petering (4) have reported synthesis of 5-bis(2-chloroethyl) amino uracil (U-8344). Antitumor activity of the compound was demonstrated against 2 rat tumors, Walker adenocarcinoma 256 and the Murphy-Sturm lymphosarcoma. This report describes antitumor activity of U-8344 in 2 mouse tumors, Sarcoma 180 and Ehrlich carcinoma. Chlorambucil (CB-1348) was included in the tests as a reference compound.

Experimental. The acute LD_{50} in Swiss mice for U-8344 and chlorambucil (CB-1348) was 3.5 mg/kg and 63.5 mg/kg, respectively, when the drugs were given in a single intraperitoneal injection and animals observed for 7 days. U-8344 was screened against the ascitic form of Ehrlich carcinoma.† Groups of 10 female Swiss mice were implanted intraperitoneally with 4.2 million tumor cells. Treatment was started 16-20 hours later. The drug was dissolved in 95% ethyl alcohol and stored at 4°C. Solutions were prepared daily for injection by diluting aliquots with 1% carboxymethyl cellulose. Injections were

TABLE I. Effect of U-8344 against Ehrlich Carcinoma (Ascites).

		8th day		13th day	
	Dosage (mg/kg/day)	Change in body wt (g)	Deaths	Change in body wt (g)	Deaths
Tumor control		+ 8	0	+ 14	1
Non-tumor control		+ 2	0	+ 3	0
U-8344	.8	+ 4	0	+ 9	0
"	.4	+ 4	0	+ 8	2
"	.2	+ 5	0	+ 12	1
"	.1	+ 9	0	+ 16	3

TABLE II. Effect of U-8344 and Chlorambucil on Survival of Mice with Ehrlich Ascites.

Preparation	Dosage (mg/kg/day)	Median days	Survival time (stand. error)	P*	Alive at 82 days
U-8344	.2	19	(4.0)	.50	2
	.4	34	(3.8)	<.01	5
	.8	43	(7.9)	<.01	5
	1.2	30	(6.1)	.02	2
	1.6	27.5	(4.7)	.02	2
Chlorambucil	5.0	16.5	(1.3)	.76	0
	10.0	19.0	(1.4)	.07	0
	20.0	12.5	(2.2)	.13	0
Tumor control		16.0	(0.9)		0
Non-tumor control					16

* P = Approximate probability of observed deviation from control median ST (normal deviate test).

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TABLE III. Effect of U-8344 and Chlorambucil on TPCV of Ascitic Tumor.

Preparation	Dose, mg/kg/day	Ehrlich ascites					Sarcoma 180 ascites				
		No. survivors	No. no cells	P*	Avg fluid vol, ml	TPCV, ml Avg	TPCV, ml S.E.	No. survivors	No. no cells	P*	Avg fluid vol, ml	TPCV, ml Avg	TPCV, ml S.E.
Tumor control		36	1		7.59	2.01	.13	30	1		6.18	1.65	.09
Chlorambucil	5	12	0	.56	3.29	1.05	.12	14	0	.69	3.93	1.04	.07
	10	15	13	<.01	.35	.08	.07	15	6	<.01	1.10	.36	.09
	20	15	14	"	.33	.09	.09	15	14	"	.77	.03	.03
U-8344	.1	15	2	.42	4.61	1.07	.14	15	0	.73	5.35	1.26	.08
	.33	15	14	<.01	.23	.06	.06	15	15	<.01	.0	<.01	
	1	13	13	"	.0	<.01		14	14	"	.0	"	

* P = Probability of chance deviation from control in No. with no cells as large as observed deviation (Chi-square test).

made subcutaneously twice daily for 7 days. Mice were weighed at the start of the experiment and on T_8‡ and T_{13}. U-8344 in doses of 0.4 to 0.8 mg/kg/day produced a 50% inhibition (T_8) of body weight gain of ascitic mice (Table I). Inhibition of development of ascitic fluid is noted also on the 13th day.

U-8344 was also tested against the Ehrlich carcinoma using survival as the index of activity. Chlorambucil was used as a standard drug for comparative purposes. Solutions of U-8344 and chlorambucil were made in ethyl alcohol. Dilutions were made daily with saline. The drugs were injected intraperitoneally once daily for 3 days into groups of 20 female mice which had been implanted with 4.0-4.5 million tumor cells. Survivors were checked daily. The experiment was terminated on the 82nd day. Average survival time was calculated by assuming that survivors died 24 hours after termination date. Approximately 30% of survivors in all groups showed solid tumors at site of injection. U-8344 was more effective than chlorambucil in prolonging survival time of mice with Ehrlich carcinoma in the doses used. (Table II).

U-8344 was tested against the ascitic forms of Ehrlich carcinoma and Sarcoma 180, using the total packed tumor cell volume (TPCV) technic. The drugs were dissolved in ethyl alcohol and diluted daily with saline. Injections were made once daily for 7 days, beginning 20 hours after implanting the tumor. Total packed tumor cell volume was determined 24 hours after last injection. Fifteen mice were used per dosage level; 40 mice were used for Ehrlich and 30 were used for Sarcoma 180 ascites controls. U-8344 is effective against the 2 ascitic tumors and is more effective than chlorambucil on either a weight or molar basis (Table III).

One-tenth ml of a homogenate of Sarcoma 180 was implanted subcutaneously in

† The Ehrlich carcinoma (ascites form) was obtained from Dr. T. S. Haushka, of Roswell Park Memorial Inst. and has been carried in our laboratory since May 1956. Sarcoma 180 was obtained from Dr. K. Sugiura, of Sloan-Kettering Memorial Inst. and has been carried in our laboratory since Jan. 1957.

‡ T_8 refers to 8th day after transplanting tumor.

TABLE IV. Effect of U-8344 against Sarcoma 180.

Preparation	Dosage (mg/kg/day)	Change in body wt (g)	Tumor wt mg	(S.E.)	P*	% tumor inhibition	Deaths
U-8344	.2	+ 1	474	(52.3)	.32	13	0
	.4	0	281	(48.2)	.05	49	0
	.8	- 3	94	(16.1)	<.01	83	0
Tumor control		+ 3	546	(142.2)			0
U-8344	.8	- 5	67	(11.9)	.01	69	0
	1.2	- 4	29	(7.2)	<.01	87	2
	1.6	-10	15	(2.4)	<.01	93	7
Tumor control		0	218	(54.6)			0

* P = Probability of observed deviation from control avg (t-test).

TABLE V. Effect of Dosage Schedule on Inhibition of Sarcoma 180 by U-8344.

Preparation	Dosage* (mg/kg/day)	Dosage schedule	Change in body wt (g)	Tumor wt mg	(S.E.)	P†	% tumor inhibition	Deaths
U-8344	.8	T_1, T_4, T_7‡	+ 1	338	(54.2)	.05	41	1
	1.2	"	- 1	200	(21.4)	<.01	65	1
	1.6	"	- 7	172	(41.6)	"	70	1
Tumor control			+ 3	571	(123)			0
U-8344	.8	T_5, T_8, T_{11}‡	0	770	(105.3)	.03	36	0
	1.2	"	- 4	743	(161.0)	.04	38	0
	1.6	"	- 8	717	(69.1)	.02	40	2
Tumor control			- 2	1200	(171.9)			2
U-8344	.8	T_1-T_7§	- 4	128	(14.5)	<.01	76	0
	1.2	"	- 5	79	(8.2)	"	85	1
	1.6	"	- 7	37	(7.6)	"	92	1
Tumor control			0	596	(54.9)			0

* Drug was given intraper. once daily, diluted in 0.9% NaCl.
† Probability of observed deviation from control avg (t-test).
‡ Sacrificed 4 days after last inj.
§ " 24 hr " " ".

the groin of Swiss mice. Treatment was started 16-20 hours after tumor implantation and continued daily for 7 days. The tumors were dissected out and weighed on a Roller-Smith balance 16-20 hours after last injection. Drugs were given intraperitoneally in single daily doses in saline. Ten mice were used on each dosage level.

A significant inhibition of tumor weight was observed when 0.8 mg of U-8344 was given per kg of body weight (Table IV).

A preliminary study was made to determine if U-8344 was effective on established Sarcoma 180 tumors. It was found (Table V) that U-8344 was less effective on 5-day-old Sarcoma 180 tumors using dosage schedules that produced significant inhibition of 1-day tumors. Drug levels used produced severe body weight loss. Inanition of the animals may be responsible for a significant portion of the tumor inhibition.

Conclusion. 5-Bis(2-chloroethyl) amino uracil (U-8344) is an effective agent against the solid and ascites forms of Ehrlich carcinoma and Sarcoma 180 under specified conditions.

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