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Further Evaluation of Bimolane and Analogs as Potential Antitumor Agents*

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Abstract—*Bimolane has been shown to have good antitumor activity and on an equitoxic basis its activity is usually better than the chemically related razoxane. Resistance patterns of these two piperazinediones were similar. They exhibited cross-resistance to each other but little or no cross-resistance to most other clinically used drugs tested. Partial resistance was observed, however, between the piperazinediones and vincristine, daunorubicin and doxorubicin. The antitumor activities of three new analogs were compared with bimolane, ICRF-154 and razoxane against four rodent tumors. In general, bimolane was most effective.*

INTRODUCTION

BIMOLANE (AT-1727) [1,1′-bis(morpholinomethyl)-4,4′-(1,2-ethanediyl)bis(2,6-piperazinedione)] was synthesized by the main author in 1980 [1]. It is used clinically in the People's Republic of China for the treatment of psoriasis, uveitis, several types of neoplastic disease and as a radiation-potentiator. It is a derivative of ICRF-154, synthesized by replacing the hydrogen in the imino groups with a morpholinomethyl group so that another part, possibly with alkylating ability, might be added to ICRF-154. The compound not only maintains excellent antitumor activity, but also gives a higher chemotherapeutic index than ICRF-154 against sarcoma S-37 [2]. Recent experimental studies in animals indicate that bimolane exhibits significant antitumor activity [1–3]. Comparisons with the clinically used drug razoxane [4, 5] led us to investigate bimolane further, especially with respect to drug resistance, and to try to improve its effect by the synthesis of more water-soluble analogs (Fig. 1). Since morpholine itself possesses no significant biological activity, we substituted morpholine in bimolane with the biologically active compounds, maleimide, which is a mercapto-combining agent, maleic hydrazide, a plant growth inhibitor with slight antitumor activity, or 2-amino-1,3,4-thiadiazole, an active antitumor agent itself [6].

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MATERIALS AND METHODS

Chemicals

The piperazinediones used in this study were synthesized in our laboratory; other compounds were obtained from the Drug Development Branch, Drug Research and Developmental Chemotherapy, NCI, Bethesda, MD, U.S.A.

The three new piperazinediones were synthesized by the following general procedure: to a boiling mixture of 4,4′-(1,2-ethanediyl)bis(2,6-piperazinedione) (0.01 M) and maleimide, maleichydrazide or 2-amino-1,3,4-thiadiazole (0.021 M), suspended in a mixture of DMF (20 ml) and ethyl alcohol (5 ml), a 37% formaldehyde solution (6 ml, 0.06 M) was added portionwise until the mixture became clear. The solution was cooled and filtered. Ethyl ether was added until the filtrate became slightly turbid and the mixture was refrigerated overnight. The white solid that separated was collected and dried *in vacuo* and gave satisfactory elemental analyses. The structures were confirmed by NMR spectra. The maleimido- and thiadiazolo-analogs were dissolved in 0.9% NaCl solution; the others were suspended in 0.5% carboxymethylcellulose in 0.9% NaCl solution. Bimolane and razoxane were stored frozen in daily samples at about −20°C. The other analogs were prepared immediately prior to injection.

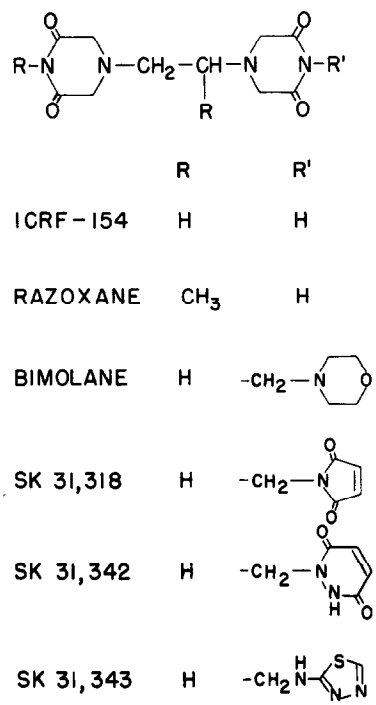

Fig. 1. Structures of bimolane and analogs. ICRF-154, 4,4'-(1,2-ethanediyl)bis(2,6-piperazinedione); razoxane, ICRF-159, (±)-4,4'-(1-methyl-1,2-ethanediyl)bis(2,6-piperazinedione); bimolane, 1,1'-bis-(morpholinomethyl)-4,4'-(1,2-ethanediyl) bis(2,6-piperazinedione); SK 31,318, 1,1'-bis(maleimidomethyl)-4,4'-(1,2-ethanediyl)bis(2,6-piperazinedione); SK 31,342, 1,1'-bis(maleic hydrazidomethyl)-4,4'-(1,2-ethanediyl)bis(2,6-piperazinedione); SK 31,343, 1,1'-bis(2-amino-1,3,4-thiadiazolomethyl)-4,4'-(1,2-ethanediyl)bis(2,6-piperazinedione).

Animals, tumors and antitumor testing

L1210, Lewis lung carcinoma (LL) and S180 (ascites) were grown in female C57BL/6J × DBA/2 (BDF_1) mice from Harlan-Sprague-Dawley, Madison, WI, U.S.A. Ridgway osteogenic sarcoma (ROS) was grown in AKR × DBA/2 ($AKD2F_1$) from Jackson Laboratories, Bar Harbor, ME, U.S.A. In general, groups of 5-10 mice weighing 19-22 g were used.

For the development of the bimolane and razoxane-resistant L1210 sublines, the six daily maximum tolerated i.p. doses were given to successive transplant generations. Established resistant sublines were used for the cross-resistance studies.

Leukemia L1210 (10^6 cells) and S180 (10^7 cells) were transplanted i.p. on day 0. LL was implanted by means of tissue homogenate and ROS by trocar pieces of ~2 mm diameter [3, 9] s.c. into the right axillary region. Details of injection schedules are listed in the appropriate tables. The approximate maximum tolerated doses (MTD = 10% lethal dose) were used. They were obtained by plotting dose-mortality data on probability paper.

The effect of treatment on L1210 was determined by increase of the median survival time (MST), here expressed as % increase in lifespan (ILS). The effect of treatment on LL and ROS was determined on the basis of the ratio of treated (T)/control (C) of the average tumor diameters (averages of two perpendicular diameters) on day 14 or 19, respectively, and also by the ILS. S180 was evaluated on the basis of the ratio T/C of the average total packed cell volume on day 7 [7, 8].

Statistical analysis was done using Student's *t* test.

RESULTS

Bimolane and razoxane were tested against several drug-resistant L1210 sublines (Table 1). Moderate-to-full activity, as compared with the

Table 1. Effect of bimolane and razoxane on L1210-resistant sublines

L1210 and sublines resistant to:	Controls MST (days) ± S.D.	Bimolane* MST (days) ± S.D.	% ILS	Razoxane* MST (days) ± S.D.	% ILS
L1210/0	7 ± 0.3	14 ± 2.2	100	13 ± 0.7	86
Bimolane	8 ± 0.5	9 ± 1.2	12†	9 ± 1.0	12†
Razoxane	8 ± 0	9 ± 0.5	12†	9 ± 0.7	12†
Ara-C	8 ± 0	15 ± 2.1	86‡	13 ± 2.2	63‡
6-Mercaptopurine	7 ± 0.4	12 ± 2.1	71‡	11 ± 0.4	57‡
5-Fluorouracil	8 ± 0.3	14 ± 2.5	75‡	13 ± 1.1	63‡
Methotrexate	8 ± 0.5	14 ± 0.6	75‡	13 ± 0.5	63‡
Cyclophosphamide	8 ± 0.9	13 ± 1.3	63‡	12 ± 2.0	50‡
L-Phenylalanine mustard	8 ± 0.5	14 ± 1.6	75‡	14 ± 2.8	75‡
Vincristine	7 ± 0.7	9 ± 0.5	29§	9 ± 1.5	29§
Doxorubicin	8 ± 0.5	10 ± 0.5	25§	9 ± 0.8	13§
Daunorubicin	7 ± 0.5	9 ± 0.8	29§	9 ± 0.8	29§

*Inoculum was 10^6 cells i.p. on day 0. Treatment was days 1-6 i.p., bimolane 89 mg/kg, razoxane 106 mg/kg.

†$P < 0.001$.

‡$P > 0.05$.

§$P < 0.01$, as compared with parent line.

parent L1210 line, was manifest in all sublines; against some sublines bimolane was slightly more effective than razoxane. The weakest effect of both chemicals was on the vincristine-, doxorubicin- and daunorubicin-resistant sublines.

Bimolane and razoxane-resistant L1210 sublines were developed. Both lines were resistant after 5-6 treatment generations. Bimolane and razoxane showed complete cross-resistance but sensitivity of both resistant lines to other antitumor drugs were similar to the parent L1210 line [Schmid, unpublished data].

The LD_{10}s for a 6-day i.p. treatment schedule for all drugs are listed in Table 2. On a molar basis razoxane is approximately half as toxic and the maleimido analog (SK 31,318) is twice as toxic as bimolane and the other analogs.

Tables 3 and 4 show the effect of the six compounds on a 6-daily i.p. injection schedule against L1210, S180 and Lewis lung carcinoma, and Ridgway osteogenic sarcoma. The doses used were molar multiples up to the maximum tolerated dose. In all tumor systems bimolane had the greatest effect but SK 31,342 (the maleic hydrazido analog) was as effective as razoxane and ICRF-154.

Table 2. Maximum tolerated doses (mg/kg) *of bimolane and analogs in female BD2F$_1$ mice*

	Bimolane	Razoxane	ICRF-154	SK 31,318	SK 31,342	SK 31,343
6 daily i.p. doses	89	106	55	55	80	95
Single i.p. dose	330	>1000	400	290	270	285
Molecular weights	452	268	254	472	502	480

*Table 3. Comparative effect of bimolane and analogs against L1210 leukemia and S180 ascites**

Treatment days 1-6 i.p.: drug, mg/kg	L1210 (10^6 cells i.p.) Day 6 AWC (g)	MST ± S.D. (days)	% ILS	S180 (10^7 cells i.p.) Day 7 carcass AWC (g)	ATPCV ± S.D. (ml)	ATPCV T/C
Bimolane						
89	-1.0	16 ± 2.1	129	0.0	0.01 ± 0.03	0.01
44	+1.8	12 ± 1.3	71	0.0	0.37 ± 0.60	0.29
22	+2.8	11 ± 8.3	57	-1.3	1.27 ± 0.57	1.00
11	+2.5	10 ± 0.5	43			
Razoxane						
106	+0.1	14 ± 2.2	100	+0.1	0.23 ± 0.04	0.18
53	+0.7	12 ± 1.5	71	+0.7	0.98 ± 0.15	0.77
13	+2.8	10 ± 0.5	43			
ICRF-154						
50	+0.3	15 ± 1.5	114	-0.3	1.20 ± 0.54	0.94
25	-1.8	10 ± 1.3	43	-1.3	1.38 ± 0.24	1.08
SK 31,318						
46	-1.0	12 ± 3.7	71	-1.6	0.37 ± 0.17	0.29
23	+2.6	11 ± 0.4	57	-0.2	0.76 ± 0.26	0.60
SK 31,342						
100	-2.2	15 ± 5.6	114	-2.2	0.05 ± 0.07	0.04
50	+2.0	11 ± 0.6	57	+2.0	0.38 ± 0.21	0.30
25	+3.8	15 ± 5.6	57	+3.8	1.23 ± 0.10	0.97
SK 31,343						
95	+3.6	11 ± 0.5	57	-1.2	0.06 ± 0.23	0.05
48	+2.4	10 ± 0.5	43	-1.8	0.36 ± 0.27	0.28
24	+1.4	9 ± 0.6	29	-2.4	0.84 ± 0.04	0.66

AWC = average weight change; MST = median survival time; ILS = increase in lifespan; T/C = treated/control; ATPCV = average total packed cell volume.

*Tumor transplantation was on day 0. Treatment was started on day 1. There were 5-15 BDF$_1$ female mice/group.

Table 4. Comparative effect of bimolane and analogs against Lewis lung carcinoma (LL) and Ridgway osteogenic sarcoma (ROS)

Treatment days 1-6 i.p. drug, mg/kg	LL (~5 × 10^5 cells s.c.) Day 14 AWC (g)	ATD ± S.D.	ATD T/C	% ILS	ROS (Trocar flank s.c.) Day 19 AWC (g)	ATD ± S.D.	ATD T/C	% ILS
Bimolane								
89	-1.1	0.50 ± 0.30	0.39	63	-3.5	0.71 ± 0.35	0.29	43
44	+1.6	1.16 ± 0.19	0.90	25	+1.9	1.22 ± 0.15	0.50	112
22	+2.3	1.16 ± 0.20	0.90	16	+4.8	1.96 ± 0.19	0.81	31
Razoxane								
106	+3.0	1.13 ± 0.30	0.88	10	-2.2	1.18 ± 0.33	0.49	46
53	+3.1	1.27 ± 0.32	0.99	0	+2.4	1.52 ± 0.21	0.63	23
ICRF-154								
50	-0.8	0.95 ± 0.08	0.74	44	-1.1	1.13 ± 0.46	0.47	51
25	+1.4	1.16 ± 0.30	0.91	9	+5.1	2.03 ± 0.31	0.84	39
SK 31,318								
46	+3.5	1.27 ± 0.19	0.99	0	+0.5	1.33 ± 0.34	0.55	71
SK 31,342								
100	-3.0	0.52 ± 0.16	0.40	31	-2.6	1.01 ± 0.23	0.42	39
50	+0.7	1.08 ± 0.32	0.84	9				
25	+1.0	1.24 ± 0.24	0.97	0	+4.9	1.88 ± 0.31	0.78	10
SK 31,343								
95	+2.2	1.25 ± 0.28	0.98	9	+2.3	1.32 ± 0.33	0.55	43
48	+2.7	1.11 ± 0.18	0.87	6	+6.5	2.21 ± 0.39	0.91	39
24	+0.6	1.20 ± 0.10	0.93	6	+6.7	2.23 ± 0.30	0.92	39

AWC = average weight change; ATD = average tumor diameter; T/C = treated/control; % ILS = increase in lifespan.

*Tumor transplantation was on day 0. Treatment was started on day 1. There were 5-10 female mice/group (LL in BDF_1; ROS in $AKD2F_1$).

DISCUSSION

The development of drug resistance is the most important single problem in cancer chemotherapy. Therefore, the relatively non-cross-resistant profile of the piperazinediones is a desirable characteristic of a good drug. It is for these reasons that we extended our studies on bimolane into the field of drug resistance and into the synthesis of new piperazinediones with hopefully greater antitumor activity. Cross-resistance among vincristine and anthracyclines is well documented [9, 10]. Apparently the primary determinant of resistance of these cells is altered plasma membrane permeability to the drugs. It thus appears that the partial cross-resistance to bimolane and razoxane is associated with the same multidrug-resistant phenotype which resides at the membrane level.

The antitumor activities of bimolane and other analogs were compared on an equitoxic basis against four tumors. In general, bimolane and the maleic hydrazido analog had slightly better activity.

On the basis of these data, piperazinediones appear to have good antitumor activity and their non-cross-resistant profile makes them a good choice for drug combination protocols.

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