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Antitumor Activity and Toxicity in Animals of RR-150 (7-Cysteaminomitosane), a New Mitomycin Derivative

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ABSTRACT

The experimental antitumor activity of a new mitomycin derivative, 7-cysteaminomitosane (RR-150), was evaluated in mice. When administered i.p. to mice bearing i.p.-implanted tumors, RR-150 was superior to mitomycin C (MMC) in increasing the life span of animals bearing P388 leukemia, B16 melanoma, and a line of L1210 leukemia partially resistant to MMC. RR-150 appeared comparable to MMC in increasing life span of mice bearing Madison 109 lung carcinoma, Colon 26 carcinoma, or parental (nonresistant) L1210 leukemia. Mice immunosuppressed with 550 rads whole-body irradiation prior to i.p. implantation of B16 still benefited (*e.g.*, 40% cure rate) following optimal RR-150 therapy when compared to nonirradiated, B16-implanted mice given RR-150 (*e.g.*, 70% cure rate). RR-150 had inconsistent activity in the treatment of s.c.-implanted tumors.

In toxicity evaluations, RR-150 was comparable to MMC in suppression of total white blood cell counts but appeared to be less neutropenic. RR-150 also caused less cumulative leukopenia than did MMC in a weekly chronic dose experiment. Based on serum chemistries, RR-150 did not have significant nephrotoxicity, but there was evidence of possible liver toxicity at doses near the 50% lethal dose.

Because of the balance of favorable antitumor and toxicity properties of RR-150, work is under way to prepare a more bioavailable form for advanced evaluation.

INTRODUCTION

MMC[2] is a highly active antitumor antibiotic in both animal tumor systems and a variety of human neoplastic diseases. Its utility in human therapy, however, has been limited primarily by severe cumulative myelosuppression (8). Recent attempts to circumvent this toxicity with autologous bone marrow transplantation have uncovered other side effects (17, 22), and the results suggest further improvement in MMC utility may be limited. Thus, there is a continuing effort which has extended over many years to prepare analogues of MMC with improved therapeutic effectiveness (10, 11, 13–15). More recently, emphasis has been directed toward reducing myelosuppression as well as maintaining or increasing antitumor efficacy (1, 5, 11). Structure-activity relationships indicated: (*a*) the intact mitosane structure was necessary to maintain potency and antitumor effectiveness; (*b*) 1A alkyl-substituted derivatives (porfiromycin type) offered no advantage and usually lost potency; and (*c*) leukopenia, antitumor activity, and potency did not parallel each other from one analogue to the next (10–13). Attention was thus focused on preparation of substituted amines at position 7. From over 200 analogues prepared, RR-150 (Chart 1) appeared to have the most favorable biological properties in initial screening (6, 12). Its antitumor activity and toxic side effects on murine models are reported here.

MATERIALS AND METHODS

Animals. BALB/c, DBA/2, C57BL/6, BALB/c × DBA/2 F_1 (hereafter called $CD2F_1$) and C57BL/6 × DBA/2 F_1 (hereafter called $BD2F_1$) mice of both sexes, 16 to 20 g, were used for antitumor testing. Male $BD2F_1$ mice, 25 to 29 g, were used for toxicity studies. All mice were obtained from either Charles River Breeding Laboratories, Inc. (Wilmington, MA), Taconic Farms, Inc., (Germantown, NY), or Lab Supply Co., Inc. (Indianapolis, IN).

Drugs. RR-150 (NSC 329697) was suspended in 0.9% NaCl solution (saline) following initial dissolution in a small amount of dimethyl sulfoxide. MMC from clinical vials containing mannitol:MMC (2:1) was diluted directly with saline. All injections were i.p. except as otherwise indicated. The structures of RR-150 and MMC are shown in Chart 1.

Irradiation. WBI was administered using a gamma cell-40 ^{137}Cs source (Atomic Energy of Canada, Ltd.).

Tumors. P388, L1210, and L1210/MMC leukemias were maintained in ascitic form in DBA/2 mice. DBA/2 mice used to propagate L1210/MMC were treated on the first 3 days following tumor transplantation with MMC (0.8 mg/kg/day) in order to maintain selective pressure (18). B16, M109, and C26 were maintained as s.c. growing tumors in C57BL/6 (for B16) or BALB/c (for M109 and C26) mice. Chemotherapeutic studies were performed in hybrid animals histocompatible through one parent with the mouse strain of tumor origin.

Chemotherapy Tests. Experiments involving L1210, L1210/MMC, or P388 were initiated by implanting mice with 10^5 or 10^6 leukemia cells. There were 6 mice in each drug treatment group and 10 mice in vehicle-treated control groups.

B16 experiments were begun by implanting 0.5 ml of a 10% tumor brei suspension i.p. or 25 mg (approximately) of tumor fragments s.c. (via trocar) into $BD2F_1$ mice. Drug-treated and tumor control groups consisted of 10 mice. M109 and C26 experiments were begun by implanting 0.5 ml of a 2% (M109) or 1% (C26) tumor brei suspension i.p. into $CD2F_1$ mice. Both drug-treated and tumor control groups consisted of 8 mice.

WBI, 550 R, was used to immunosuppress $BD2F_1$ mice 2 days prior to i.p. implantation of B16. Such irradiated mice were used to evaluate the relative therapeutic potentials of RR-150 and MMC compared to their effects in concomitantly nonirradiated B16-bearing mice.

L1210 and P388 experiments were terminated on Day 30, and B16, M109, and C26 experiments were terminated on or near Day 60. Mice alive at the end of an experiment were autopsied and judged to be cured if no signs of disease were visible. Each drug in an experiment was evaluated at a minimum of 3 dose levels for each treatment schedule

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[2] The abbreviations used are: MMC, mitomycin C; RR-150, 7-cysteaminomitosane; B16, B16 melanoma; M109, Madison 109 lung carcinoma; C26, Colon 26 carcinoma; MST, median survival time; L1210/MMC, L1210 partially resistant to mitomycin C; % T/C, median survival time of drug-treated (T) mice divided by the median survival time of tumor control mice (C) × 100; TD, tumor volume-doubling time; LD_{50}, dose required to kill 50% of the treated animals; BUN, blood urea nitrogen; SGPT, serum L-alanine aminotransferase; SGOT, serum aspartate aminotransferase; WBI, whole-body irradiation.

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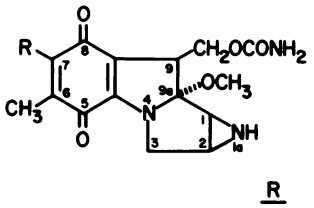

	R
RR-150	$HSCH_2CH_2NH-$
Mitomycin C	NH_2-

Chart 1. Structures of RR-150 and of MMC.

used. The various treatment schedules are described in "Results" and in Table 1. Mice were observed daily, and antitumor drug activity was determined based upon: (*a*) the proportion of mice cured; and (*b*) the % T/C.

Additionally, an attempt was made to quantitate the degree of tumor cell kill achieved with each drug regimen using methods described earlier (21) and more recently (7). Briefly, the $\log_{10}$ net cell kill achieved was calculated as

$$\text{Log}_{10}\text{ net cell kill} = \frac{\text{T–C value (days)} - \text{duration of treatment (days)}}{3.32\ (\text{TD})}$$

where T–C value was the MST for drug-treated dying mice only, minus the MST for control mice. Such calculations were performed only with data from experiments where drug activity (as defined above) was observed. If the $\log_{10}$ net cell kill was positive, there were fewer cells present at the end of therapy than at the start. The TDs of the various tumor models used for the calculation of net tumor cell kill were: i.p. and i.v. P388, 0.57 day; i.p. L1210, 0.45 day; i.p. L1210/MMC, 0.4 day; i.p. B16, 1.4 days; s.c. B16, 1.3 days; i.p. M109 and i.p. C26, 1.2 days. The TDs for P388 and L1210 leukemias were estimated from a best-fit straight line from a log-linear plot of the MST (of dying mice only) *versus* $\log_{10}$ dilutions of the leukemia cells in untreated mice. The TD values for M109 (19) and B16 (9) were obtained from the literature, and TD values for C26 were from unpublished data.

Drug-treated mice dying prior to the first death among parallel, untreated, tumor control mice were presumed to have died from drug toxicity and were excluded from calculations of MST. No result of therapy is reported in which deaths attributable to drug toxicity exceeded 17% in the treated group (*i.e.*, allowing for one early death among a group of 6 mice).

Toxicity Testing. An acute LD_{50} was determined for RR-150 and MMC following single i.p. injections into mice (groups of 10 mice/dose). The mice were observed for 30 days, and the LD_{50} values were calculated according to the method of Weil (23).

Hematology and serum chemistry studies were performed using methods which have been described previously (4, 5). Three groups of mice, 35 to 40/group, were bled from the retroorbital plexus 3 days prior to dosing to determine their individual pretreatment total WBC and differential leukocyte counts (Group 1), BUN and creatinine levels (Group 2), or SGPT and SGOT levels (Group 3). The WBC counts were made using a Model S Coulter Counter (Coulter Electronics, Inc., Hialeah, FL), and the differential leukocyte counts were done manually. The serum chemistry values were determined using a Centrifichem System 400 (Union Carbide Co., Rye, NY). RR-150 was administered as a single i.p. injection at several dose levels (5 to 10 mice/dose) based on the optimum dose from P388 experiments (hematology study) or the single-dose LD_{50} (serum chemistry studies). MMC was included in the hematology study for comparison.

The hematology measurements were determined 3, 5, and 7 days after drug administration in the acute toxicity (single-dose) experiment and every fourth and seventh day in the chronic toxicity (one dose weekly for 5 injections) experiment. Preliminary experiments with MMC have shown a pattern of maximum leukopenia between 3 and 5 days with some recovery at 7 days after each dose (1, 5). Thus, in order to illustrate the trend of cumulative leukopenia, the data are presented graphically in the charts as the nadir day only. A decrease in the total WBC of ≥35% relative to the pretreatment count was considered indicative of a drug-induced reduction. The BUN and creatinine measurements were determined 4, 7, and 11 days and the SGPT and SGOT measurements were determined 1, 3, and 7 days after drug dosing, respectively. BUN values >30 mg/100 ml, creatinine, >0.8 mg/ml, and SGPT or SGOT increases of >200% relative to pretreatment values were considered indicative of drug-induced toxicity.

RESULTS

Antitumor Effects. RR-150 was evaluated against a number of murine tumors. The optimum doses and maximum therapeutic effects achieved in each are summarized in Table 1.

Against P388 leukemia, RR-150 showed consistently high activity, whereas results with MMC were variable. Against L1210 leukemia, RR-150 was about comparable to MMC in activity and showed little evidence of schedule dependency based on increases in survival. The effect of altering regimens of MMC is consistent with those reported previously (16). In a single test against L1210/MMC, RR-150 maintained a high degree of effectiveness (% T/C = 171), while the effect of MMC was borderline (% T/C = 129).

Among the tumors tested, B16 melanoma demonstrated the most striking sensitivity to RR-150 with cure rates (tumor-free survivors, Day 60) as high as 90%. An independent experiment performed at the laboratories of Arthur D. Little, Inc. (Cambridge, MA), confirmed these results. The effectiveness of RR-150 is best illustrated by survival curves of a typical experiment showing results with optimum and half-optimum doses of RR-150 and MMC (Chart 2). In an experiment with B16 (Table 1) in which the start of therapy was delayed for 5 days (Treatment Days 5, 9, and 13), and effectiveness of RR-150 dropped sharply (T/C = 164). The activities of RR-150 and MMC in immunosuppressed (550 rads WBI) mice bearing B16 were nearly as good as those observed in nonirradiated mice (Table 2).

Rather inconsistent results were observed in tests with M109 carcinoma. With both M109 and C26 tumors, the effectiveness of RR-150 and MMC were considered about equivalent (Table 1).

In other experiments (data not shown), RR-150 was administered parenterally (i.p. or i.v.) to mice with s.c. implanted B16. Effects with RR-150 were considered inconsistent since 2 of 3 such experiments were negative. MMC is usually moderately effective in this system.

Toxic Effects. The single-dose i.p. LD_{50} of RR-150 was 23.4 mg/kg, and that of MMC was 7.5 mg/kg in male $BD2F_1$ mice. The effect of these drugs on total and differential WBC counts is shown in Table 3. Both drugs caused reduction in WBC and lymphocyte counts at or above the optimum dose for P388 therapy. However, RR-150 did not cause reduction in neutrophils, whereas MMC caused neutropenia. When these drugs were given chronically once weekly for 5 injections, RR-150 appeared to cause less cumulative leukopenia at maximum

Table 1

Comparison of the antitumor activity of RR-150 with that of MMC

Tumor inoculum and route	RR-150				MMC			
	Optimum dose i.p. (mg/kg/injection)	Treatment schedule	Maximum % T/C[a]	Net $\log_{10}$ cell kill[b]	Optimum dose i.p. (mg/kg/injection)	Treatment schedule	Maximum % T/C	Net $\log_{10}$ cell kill
P388, 10^6, i.p.	12.8	Day 1	313 (2/6)[c]	>6	1.6	Day 1	181	2.9
P388, 10^6, i.p.	12.8	Day 1	306	>6	6.4	Day 1	320	>6
P388, 10^6, i.p.	12.8	Day 1	>356 (3/6)	5.8	3.2	Day 1	211	4.8
L1210, 10^5, i.p.	9.6	Day 1	169	3.0	4.8	Day 1	163	2.7
L1210, 10^5, i.p.	6.4	Days 1, 5, and 9	181	1.7	3.2	Days 1, 5, and 9	169	2.3
L1210, 10^5, i.p.	3.2	Days 1 and 9	163	2.7	1.6	Days 1 and 9	163	2.7
L1210, 10^6, i.p.	6.4	Days 1, 4, and 7	200	0.7	2.4	Days 1, 4, and 7	208	0.3
L1210/MMC, 10^6, i.p.	6.4	Days 1, 4, and 7	171	1.5	3.2	Days 1, 4, and 7	129	3.8
B16, 0.5 ml of a 10% brei, i.p.	3.2	Days 1, 5, and 9	>280 (8/10)	5.8	3.2	Days 1, 5, and 9	235 (1/10)	3.9
B16, 0.5 ml of a 10% brei, i.p.	3.2	Days 1, 5, and 9	>298 (9/10)	>6.0	3.2	Days 1, 5, and 9	256 (2/10)	4.3
B16, tumor brei, i.p. (ADL)[d]	6.0	Days 1, 4, and 7	>218 (9/10)	ND	3.0	Days 1, 4, and 7	193	ND
B16, 0.5 ml of a 10% brei, i.p.	6.4	Days 5, 9, and 13	164	1.1	1.6	Days 5, 9, and 13	145	0.2
M109, 0.5 ml of a 2% brei, i.p.	12	Days 1 and 4	135	0.6	4	Days 1 and 4	276 (1/10)	6.0
M109, 0.5 ml of a 2% brei, i.p.	9	Days 1 and 4	184	2.4	2	Days 1 and 4	156	1.3
C26, 0.5 ml of a 1% brei, i.p.	6	Days 5 and 8	137 (1)	1.4	1	Days 5 and 8	127	0.8

[a] Long-term survivors.
[b] Calculated as described in "Materials and Methods" and in Refs. 7 and 19.
[c] Numbers in parentheses, long-term survivors per total.
[d] ADL, Arthur D. Little, Inc., Cambridge, MA; ND, not done.

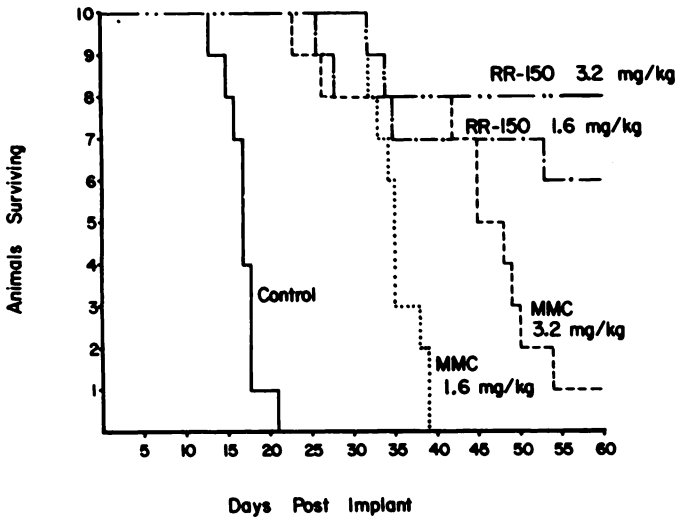

Chart 2. Effect of optimum doses of RR-150 and MMC on B16 melanoma implanted i.p. as a tumor brei (Experiment 277). Treatment given i.p. on Days 1, 5, and 9.

Table 2

Relative therapeutic effects of MMC and RR-150 against B16 melanoma implanted in normal and immunosuppressed mice

Drug	Dose (mg/kg/injection)[a]	% T/C[b]	
		Normal B16-bearing mice	WBI B16-bearing mice[c]
MMC	4	Toxic	Toxic
	3	190	185
	1	205	155
RR-150	6	310 (7/10)[d]	310 (4/10)
	4	310 (4/10)	230 (3/10)
	2	142	170
	1	170	175
	0.5	172	170
None		100	85[e]

[a] Drugs were given i.p. on Days 1, 5, and 9 following i.p. implant of 0.5 ml of a 10% B16 tumor brei.
[b] "Cures" were tumor-free mice surviving to Day 62 post-tumor implant.
[c] Mice received 550 rads of WBI 2 days prior to tumor implantation. Irradiated mice not implanted with B16 survived until termination of the experiment on Day 62 (data not shown).
[d] Numbers in parentheses, number of cures per total.
[e] Compared to MST of nonirradiated tumor control mice.

nonlethal doses (Chart 3). In studies of effects on serum chemistries, RR-150 had a marginal effect on BUN levels (one incident of BUN >30 mg/100 ml) and no effect on creatinine levels at doses up to the LD_{50} (Table 4). The SGPT and SGOT levels were increased on Day 3 by RR-150 at doses of 23.4 and 17.6 mg/kg (Table 5). The effect on SGPT was more marked. The SGPT and SGOT values were near control levels by Day 7 (data not shown).

DISCUSSION

RR-150 is one of a new generation of MMC analogues found to have superior antitumor effects in one or more tumor systems. Such a finding is not uncommon with mitomycins [see also the work of Sakurai (20)] and occurs far more frequently than with other chemotypes such as anthracyclines (2) or platinums (3). RR-150 was found to be superior to MMC against P388 leukemia

Table 3

Effect of RR-150 on total WBC, neutrophil, and lymphocyte counts

Male BD2F$_1$ mice, 10/dose, received single i.p. injections of RR-150 or MMC on Day 0.

Material	Dose (mg/kg i.p.)	Maximum % change in [a] Total WBC	Neutrophils	Lympho-cytes	Deaths by Day 7
RR-150	25.6	−60	53	−78	1/5
	12.8[b]	−29	6	−35	0/5
	6.4	−21	−24	−23	0/5
	3.2	−3	8	−7	0/5
MMC	6.4	−53	−61	−51	1/5
	3.2[b]	−38	−45	−35	0/5
Vehicle		−11	5	−15	0/5

[a] Determined on Day 3.
[b] Optimum dose from P388 therapy experiments.

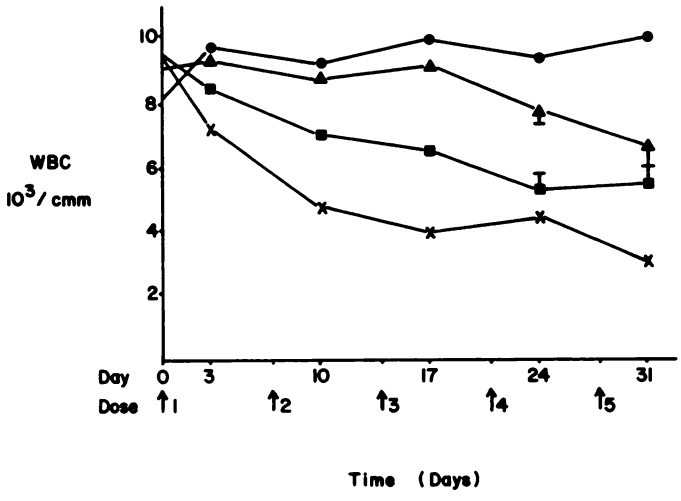

Chart 3. Effect of 5 weekly injections of RR-150 or MMC on WBC. ●, control; ▲, RR-150 (3.2 mg/kg); ■, RR-150 (6.4 mg/kg); ×, MMC (3.2 mg/kg). Each group contained 10 mice treated i.p. Measurements on nadir day for leukopenic effects.

Table 4

Effect of RR-150 on BUN and creatinine in BD2F$_1$ mice

Male BD2F$_1$ mice, 10/dose, received single i.p. injections of RR-150 on Day 0.

Dose of RR-150 i.p. (mg/kg)	Highest incidence of BUN ≥30 mg/100 ml	Creatinine ≥0.8 mg/100 ml	Deaths by Day 11
23.4[a]	1/8[b] (7)[c]	0/7 (7)	4/10
17.6	0/10 (4)	0/10 (4)	0/10
13.2	0/10 (4)	0/10 (4)	0/10
Control	1/10 (4)	0/10 (4)	0/10

[a] LD_{50}.
[b] Number of incidences per total.
[c] Numbers in parentheses, day of determination.

and B16 melanoma, possibly superior against a line of L1210 leukemia partially resistant to MMC and comparable to MMC against L1210 leukemia (parent line), M109 carcinoma, and C26 colon carcinoma. RR-150 has also demonstrated superior activity (to MMC) in a number of human tumor cell lines *in vitro* as well as greater potency in DNA cross-linking.[3] It has not, however, shown reproducible activity *in vivo* when a tumor has been implanted at a site distant from that of the therapy. Since RR-150 is poorly soluble in water (about 0.5 mg/ml), it would be essential to develop a more bioavailable dose formulation or derivative before proceeding further in development of the compound.

[3] M. G. Brattain, Baylor College of Medicine, personal communication.

Table 5

Effect of RR-150 on SGPT and SGOT

Male BD2F$_1$ mice, 10/dose, received single i.p. injections of RR-150 on Day 0.

Dose of RR-150 i.p. (mg/kg)	Maximum % of increase in SGPT	SGOT	Deaths by Day 7
23.4[a]	1164 (3)[b]	361 (3)	5/10
17.6	801 (3)	271 (3)	2/10
13.2	166 (3)	24 (7)	0/10
Control	0	87 (7)	0/10

[a] LD_{50}.
[b] Numbers in parentheses, day of determination.

It is possible that RR-150 displayed a therapeutic advantage over MMC against, *e.g.*, B16 because of a difference in their immunosuppressive effects. Conceivably, a mildly immunogenic tumor undergoing drug-induced cell reduction could be further eliminated by immunological means, providing that the drug used did not cause severe immunosuppression. If MMC and RR-150 had equivalent cell kill (*versus* B16) potential but one of them, *e.g.*, RR-150, was substantially less immunosuppressive, then conceivably RR-150 therapy might be expected to be more effective because it permitted the host's immune system to destroy residual tumor cells. We evaluated this possibility (of differing immunosuppressive effects) by treating B16-bearing mice that had received WBI with either MMC or RR-150 and compared the therapeutic effects obtained with those occurring in B16-bearing mice that had not been irradiated. RR-150 treatment of previously irradiated mice resulted in only a slightly reduced cure rate.

Compared to nonirradiated mice, the effectiveness of MMC was also only slightly diminished in WBI mice *versus* otherwise normal B16-bearing mice. Most important, however, was the fact that the relative therapeutic advantage of RR-150 over MMC was not modified in immunosuppressed mice. These results also suggest that the difference observed between the drugs was related mostly to the cytotoxic action on the tumor rather than to host-mediated effects.

The toxicity profile of RR-150 suggests that it may be less neutropenic or cumulatively leukopenic than is MMC. However, these findings must be considered with reservation until they can be confirmed with an i.v. dose form. RR-150 did not seem to cause meaningful azotemia, but there was evidence of possible liver damage based on SGPT effects. This toxicity has not been seen with MMC (5).

In summary, the biological properties of RR-150, in particular its antitumor effectiveness in some systems compared to MMC, suggests that it (or a derivative thereof) is worthy of further development. Forms of the compound demonstrating better bioavailability are under investigation.

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