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A Fluorine-containing Anthracycline (ME2303) as a New Antitumor Agent against Murine and Human Tumors and Their Multidrug-resistant Sublines[1]

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ABSTRACT

A new fluorine-containing anthracycline derivative, ME2303, showed excellent antitumor activity against various experimental tumor models. The i.p. or i.v. administrations of ME2303 on Day 1 or on Days 1, 5, and 9 against i.p.-implanted L1210 leukemia cells rendered more than 50% of mice tumor free at wide ranges of nontoxic doses, whereas the incidence of cure obtained with Adriamycin (ADM) was less than that obtained with ME2303. ME2303 given i.p. or i.v. on Day 1 or Days 1, 5, and 9 was also effective against i.p.-implanted P388 leukemia cells, and higher incidences of cure were obtained than with ADM. ME2303 administered i.v. on Days 1, 8, 15, and 22 showed prominent antitumor activity against s.c.-implanted colon adenocarcinomas 26 and 38, Lewis lung carcinoma, B16 melanoma, and M5076 sarcoma. Against colon adenocarcinoma 26, ME2303 induced cure in 16 of 20 mice at doses of 35 to 71 μmol/kg, whereas no cure was observed with ADM. Significant growth inhibition of colon adenocarcinoma 38, Lewis lung carcinoma, B16 melanoma, and M5076 sarcoma cell lines was also observed at a dose of 18 to 106 μmol/kg.

ME2303 was effective against human and murine multidrug-resistant cells *in vitro*. For example, human myelogenous leukemia K562 resistant to ADM (K562/ADM) was only 2.8-fold more resistant to ME2303, while the cells were 200-fold more resistant to ADM when the values for the concentration of drug required for 50% inhibition of cell growth were compared. ME2303 was also more effective than ADM against human leukemia CCRF-CEM resistant to vinblastine, human ovarian carcinoma A2780 resistant to ADM, human epidermoid carcinoma KB cells resistant to colchicine, and mouse leukemia P388 resistant to ADM and vincristine. Therapeutic effects were obtained *in vivo* against ADM- and, especially, vincristine-resistant P388 leukemia. ME2303 is one of the most interesting potential antitumor agents to be studied further.

INTRODUCTION

ADM[3] is one of the most widely used chemotherapeutic agents against various human neoplasias (1). It is most effective in the treatment of acute leukemia and malignant lymphoma. Although ADM has impressive antitumor activities, the effectiveness is often limited. Tumor-bearing mice treated with ADM are not necessarily cured even at an optimal dosage. One of the major reasons for the treatment failure is supposed to be the emergence of drug-resistant tumor cell subpopulations. Strong side effects of ADM such as leukopenia, thrombocytopenia, anemia, and myelosuppression are major dose-limiting factors in clinical application, which allow the regrowth of remaining tumor cells (2, 3). In the last decade, great efforts have been made to synthesize or to isolate ADM analogues that have lower toxicity but have antitumor activity superior to that of ADM (4).

It is known that 2-fluoroglycosides are resistant to chemical hydrolysis because of the presence of the electron-withdrawing fluorine atom at the C′-2 position. On the other hand, it has been reported that the replacement of the C′-3 amino group of daunorubicin and ADM with a hydroxyl group gave strong antitumor activity with weak cardiotoxicity (5–7). Taking these points into account, several derivatives have been synthesized by Takeuchi and Umezawa (8). ME2303 (Fig. 1) is a fluorine-containing (C′-2) anthracycline derivative with modification at the C-14 position, pimelate residue and a hydroxyl group replacing the C′-3 amino group. The pimelate residue is known to increase the solubility (8).

In this paper, we show evidence that ME2303 has impressive antitumor activities against a variety of tumor models. ME2303 increased the survival time of L1210- and P388-bearing mice over a wide dosage range with marginal body weight loss, and many mice were cured. ME2303 also showed strong tumor-inhibiting activity against colon adenocarcinomas 26 and 38, Lewis lung carcinoma, B16 melanoma, and M5076 sarcoma. Interestingly, the compound was effective against human and murine multidrug-resistant tumor cells *in vitro* and *in vivo*. ME2303 is a notable new antitumor agent that warrants further examination.

MATERIALS AND METHODS

Drugs. ME2303 (M_r 704.7) was provided by Meiji Seika Kaisha, Ltd., Tokyo, Japan. Other drugs were obtained from the following sources: ADM, from Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan; VCR, from Shionogi Co., Ltd., Osaka, Japan.

Animals and Tumor Cells. Adult female BALB/c × DBA/2Cr F_1 (hereafter called $CD2F_1$) mice and C57BL/6 × DBA/2Cr F_1 (hereafter called $B6D2F_1$) mice weighing 20 to 23 g were obtained from Charles River Japan, Inc., Tokyo, Japan. L1210 leukemia, P388 leukemia, Lewis lung carcinoma, B16 melanoma, colon adenocarcinomas 26 and 38, and M5076 sarcoma were supplied by the National Cancer Institute (Bethesda, MD). The human myelogenous leukemia K562 cell line was provided by Dr. K. Ezaki of this center, and the K562/ADM cell line was established in our laboratory (9). The acute lymphoblastic leukemia cell line (CCRF-CEM) and its vinblastine-resistant subline (CEM/VLB_{100}) were provided by Dr. W. T. Beck, St. Jude Children's Hospital (Memphis, TN) (10). Human ovarian cancer A2780 and its ADM-resistant variant 2780AD were kind gifts of Dr. R. F. Ozols and Dr. T. C. Hamilton, National Cancer Institute (11). Human KB carcinoma cell line KB3-1 and its colchicine-resistant variant KBC-4 were generously provided by Dr. I. Pastan, National Cancer Institute (12).

Cell Culture and Drug Treatment. Tumor cells were maintained in culture in RPMI 1640 supplemented with 5% fetal bovine serum and kanamycin (100 μg/ml) (growth medium) (13). For the drug treatment experiments, tumor cells (2×10^4 for murine cell lines and 4×10^4 for human cell lines in 2 ml of the growth medium) were incubated for 72 h in the presence of graded concentrations and counted, as described previously (13). Three samples were used for each drug concentration. IC_{50} was determined by plotting the logarithm of the drug concentration *versus* the growth rate (percentage of control) of the treated cells (13).

Evaluation of Antitumor Activity. One-tenth ml of cell suspension in HBSS containing 10^6 P388, P388/VCR, P388/ ADM, or 10^5 L1210

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[3] The abbreviations used are: ADM, Adriamycin; ME2303, 7-*O*-(2,6-dideoxy-2-fluoro-α-L-talopyranosyl)adriamycinone-14-*O*-pimelate; VCR, vincristine; ILS, increase in life span; IC_{50}, concentration of drug required for 50% inhibition of cell growth; K562/ADM, human myelogenous leukemia K562 cells resistant to Adriamycin; CEM/VLB_{100}, human acute lymphoblastic leukemia cells resistant to vinblastine; 2780AD, human ovarian cancer A2780 resistant to Adriamycin; P388/ADM, P388 leukemia cells resistant to Adriamycin; P388/VCR, P388 leukemia cells resistant to vincristine; HBSS, Hanks' balanced salt solution.

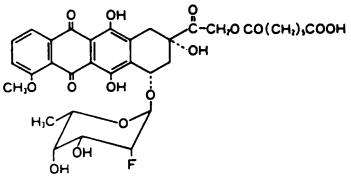

Fig. 1. Structure of ME2303.

leukemia cells was inoculated i.p. into $CD2F_1$ mice (13, 14). Cell suspensions in HBSS (20%, w/v) of Lewis lung carcinoma, B16 melanoma, colon adenocarcinomas 26 and 38, and M5076 sarcoma were prepared from surgically removed tumors. The cells were passed through 40 mesh sieves, counted for viability by trypan blue dye exclusion, and inoculated s.c. with a volume (0.2 ml) of 1×10^5 viable cells of Lewis lung carcinoma and colon adenocarcinoma 26 and of 1×10^6 cells of M5076 sarcoma into the flank of $B6D2F_1$, $CD2F_1$, and $B6D2F_1$ mice, respectively (14). Tumor cell suspensions (0.25 ml) of B16 melanoma were inoculated s.c. into the flank of $B6D2F_1$ mice. Two-tenths ml of tumor brei of colon adenocarcinoma 38 diluted in HBSS (33%, w/v) were inoculated s.c. into the flanks of $B6D2F_1$ mice (14).

Mice were given ADM, VCR, and ME2303 at a constant rate of 0.01 ml/g of body weight. Antitumor activity was determined by (*a*) comparing the mean survival time of treated group (*T*) with that of control groups (*C*) and expressing it as an increase in life span

$$\text{ILS} = \left(\frac{T}{C} - 1\right) \times 100$$

Table 1 *Antitumor effect of ME2303 and ADM administered i.p. against L1210 leukemia*

L1210 leukemia cells (10^5/mouse) were implanted i.p. into $CD2F_1$ mice (6 mice/group) on Day 0, and the drugs were administered i.p. on Day 1 or on Days 1, 5, and 9.

Drug	Dose (μmol/kg/injection)	MST[a] (days)	ILS (%)	No. of cured mice[b]	Body wt change[c] (g)
Control	0	7.7 ± 0.8[d]			+0.6
		Treatment schedule: Day 1 only			
ME2303	2.2	9.0 ± 2.4	17		+0.5
	4.4	11.4 ± 2.5	48	1/6	+0.5
	8.9	17.3 ± 5.4	125		−0.1
	18	16.3 ± 0.6	112	3/6	−0.7
	35			4/6	−0.3
	71			6/6	−0.5
	106			6/6	−2.4
	142			6/6	−3.4
	213	6.8 ± 1.0	−12	2/6	−4.2
ADM	1.7	9.7 ± 1.8	26		0
	3.4	11.3 ± 1.2	47		0
	6.9	10.7 ± 1.2	39		−1.1
	14	22.0 ± 11.0	186	1/6	−1.3
	28			4/6	−2.2
	55	7.2 ± 1.8	−6		−2.9
		Treatment schedule: Days 1, 5, and 9			
ME2303	2.2	11.5 ± 2.3	49		−0.1
	4.4	16.2 ± 6.3	110		+0.3
	8.9	17.2 ± 5.8	123	1/6	0
	18			4/6	−0.1
	35			6/6	+0.3
	71	10.0 ± 3.0	30	3/6	−1.2
	106	11.0 ± 1.2	43	2/6	−2.0
	142	11.8 ± 2.8	53		−1.3
ADM	0.4	9.7 ± 0.8	26		+0.7
	0.9	11.2 ± 2.1	45		+0.8
	1.7	11.3 ± 2.9	47		0
	3.4	11.7 ± 1.2	52		+0.6
	6.9	11.6 ± 1.3	51	1/6	−0.8
	14	13.5 ± 1.2	75		−1.2

[a] MST, mean survival time of deceased mice.
[b] Cure incidence was determined on Day 61.
[c] Difference in body weight (g) between Days 5 and 1.
[d] Mean ± SD. Cured mice were excluded from the calculations. When more than 50% of the mice were cured, the mean survival time was not calculated.

(tumor-free survivors were excluded from these calculations) or (*b*) tumor mass growth inhibition where tumor diameters were measured in two directions with calipers and the tumor volume was calculated as follows: volume = $\frac{1}{2} ab^2$; *a* is long diameter and *b* is short diameter.

RESULTS

Chemotherapeutic Effects against L1210 and P388 Leukemias. For the comparison of *in vivo* effects of ME2303 and ADM, the doses of the drugs were expressed on a molar basis (μmol/kg). The antitumor activities of ME2303 and ADM administered i.p. or i.v. on Day 1 or Days 1, 5, and 9 were examined against i.p.-inoculated L1210 leukemia (Tables 1 and 2). When the drugs were given i.p., ME2303 showed superior effects than did ADM in both treatment schedules (Table 1). All six mice were cured with ME2303 when given between 71 and 142 μmol/kg for a single injection and at 35 μmol/kg for triple injections. A single injection of ME2303 had a better therapeutic effect than the triple injections (Table 1). ADM also showed good therapeutic effect in these experiments; however, the effects were not as good as those obtained with ME2303. Four of six mice were cured with a single injection of ADM at 28 μmol/kg, which was the best treatment protocol.

When the drugs were given i.v., ME2303 again showed a superior effect than did ADM in both single and triple injections (Table 2). More than 50% of the mice were cured with ME2303 between 71 and 213 μmol/kg for a single injection and between 35 and 71 μmol/kg for triple injections. ADM showed maximal effect at 14 μmol/kg for triple injections, and two of six mice were tumor free on Day 61. These observations clearly demonstrate the superiority of ME2303 over ADM in therapeutic efficacy against L1210 leukemia.

Table 2 *Antitumor effect of ME2303 and ADM administered i.v. against L1210 leukemia*

L1210 leukemia cells (10^5/mouse) were implanted i.p. into $CD2F_1$ mice (6 mice/group) on Day 0, and drugs were administered i.v. on Day 1 or Days 1, 5, and 9.

Drug	Dose (μmol/kg/injection)	MST[a] (days)	ILS (%)	No. of cured mice[b]	Body wt change[c] (g)
Control	0	8.3 ± 1.2[d]			+0.4
		Treatment schedule: Day 1 only			
ME2303	8.9	14.7 ± 9.5	77		−0.4
	18	13.0 ± 1.5	57		0
	35	18.4 ± 3.0	122	1/6	−0.3
	71			4/6	−1.9
	106			5/6	−3.7
	142			6/6	−4.0
	213			4/6	−4.8
ADM	6.9	10.5 ± 2.1	27		−0.4
	14	14.2 ± 5.8	71		−1.9
	28	22.5 ± 9.5	171		−1.7
	55	16.2 ± 12.0	95		−4.2
		Treatment schedule: Days 1, 5, and 9			
ME2303	4.4	9.8 ± 1.6	18		+0.2
	8.9	13.5 ± 1.6	63		−0.1
	18	18.5 ± 2.3	123		+0.2
	35			6/6	−0.2
	71			6/6	−0.9
	106	9.7 ± 0.6	17	3/6	−3.4
ADM	3.4	9.3 ± 2.2	12		+0.5
	6.9	12.5 ± 7.3	51		−0.5
	14	14.8 ± 3.1	78	2/6	−0.5
	28	13.3 ± 0.8	60		−1.2

[a] MST, mean survival time of deceased mice.
[b] Cure incidence was determined on day 61.
[c] Difference in body weight (g) between Days 5 and 1.
[d] Mean ± SD. Cured mice were excluded from the calculations. When more than 50% of the mice were cured, the mean survival time was not calculated.

Table 3 *Antitumor effect of ME2303 and ADM administered i.p. against P388 leukemia*

P388 leukemia cells (10^6/mouse) were implanted i.p. into $CD2F_1$ mice (5 mice/group) on Day 0, and the drugs were administered i.p. on Day 1 or Days 1, 5, and 9.

Drug	Dose (μmol/kg/injection)	MST[a] (days)	ILS (%)	No. of cured mice[b]	Body wt change[c] (g)
Control	0	11.2 ± 1.8[d]			+0.7
		Treatment schedule: Day 1 only			
ME2303	2.2	14.0 ± 1.9	25		−0.4
	4.4	14.2 ± 3.1	27		0
	8.9	17.8 ± 3.9	59		+0.2
	18	21.0 ± 1.0	88	2/5	−1.0
	35			3/5	−0.4
	71			5/5	−1.2
	106			4/5	−3.4
	142			4/5	−2.6
ADM	1.7	12.4 ± 3.4	11		+0.8
	3.4	16.0 ± 4.0	43		+1.2
	6.9	19.2 ± 5.1	71		+0.1
	14	16.4 ± 7.4	46		−0.8
	28	18.0 ± 1.0	61	2/5	−2.0
	55	12.6 ± 6.0	13		−2.8
		Treatment schedule: Days 1, 5, and 9			
ME2303	2.2	15.6 ± 3.7	39		+0.4
	4.4	16.8 ± 5.3	50		+0.4
	8.9	20.8 ± 5.7	86	1/5	+0.2
	18			4/5	−0.2
	35			5/5	−0.6
	71	15.0 ± 2.2	34	1/5	−2.0
	106	10.6 ± 2.9	−5		−2.6
	142	11.8 ± 1.3	5		−1.4
ADM	0.4	12.8 ± 1.1	14		+0.8
	0.8	14.0 ± 2.3	25		0
	1.7	14.8 ± 3.0	32		+0.2
	3.4	16.4 ± 4.0	34		+0.8
	6.9	23.4 ± 6.2	92	1/5	+0.4
	14	15.6 ± 4.4	39		0

[a] MST, mean survival time of deceased mice.
[b] Cure incidence was determined on Day 61.
[c] Difference in body weight (g) between Days 5 and 1.
[d] Mean ± SD. Cured mice were excluded from the calculations. When more than 50% of the mice were cured, the mean survival time was not calculated.

ME2303 also showed good therapeutic activity against i.p.-inoculated P388 leukemia (Tables 3 and 4). When ME2303 was given i.p. on Day 1, more than 50% of the mice were cured when doses between 35 and 142 μmol/kg were used, and all of the mice were cured at 71 μmol/kg (Table 3). When ADM was given at the same schedule, two of five mice survived tumor free only at the most effective dose, 28 μmol/kg. ME2303 given i.p. on Days 1, 5, and 9 also induced a cure in more than 50% of the mice at doses of 18 and 35 μmol/kg, whereas ADM at the same schedule produced one tumor-free survivor of five mice at a 6.9-μmol/kg dose. Compared with ADM, a better therapeutic effect was observed for ME2303 over a wider dosage range.

When the drugs were given i.v., ME2303 showed a superior effect against i.p.-inoculated P388 leukemia than did ADM in both single and triple injections (Table 4). A single injection of ME2303 produced tumor-free survivors in two or three mice of five at doses between 71 and 213 μmol/kg, whereas a single 14- to 55-μmol/kg injection of ADM produced one tumor-free survivor of five mice (Table 4). Triple injections induced a cure in mice given doses between 35 and 142 μmol/kg of ME2303, but no cure was obtained with ADM.

Chemotherapeutic Effect of ME2303 against Colon Adenocarcinomas 26 and 38, Lewis Lung Carcinoma, B16 Melanoma, and M5076 Sarcoma. The antitumor activity of ME2303 against colon adenocarcinoma 26 was compared with that of ADM (Table 5). ME2303 given i.v. on Days 1, 8, 15, and 22 after tumor inoculation at doses 35 and 71 μmol/kg resulted in 99 to 100% inhibition of tumor growth on Day 29, and 16 of 20 mice at these doses were tumor free on Day 61. ADM, at a dose of 17 μmol/kg, showed 87% growth inhibition on Day 29, and the survival advantage of 83% ILS was obtained in this group of mice, but no cured mice were observed. In comparison with ADM, a 35-μmol/kg dose of ME2303 given i.v. induced a slight decrease (−0.3 g) in body weight; however, ADM at a dose of 34 μmol/kg caused a 2.3-g decrease in body weight.

ME2303 also showed strong tumor inhibition against colon adenocarcinoma 38, Lewis lung carcinoma, B16 melanoma, and M5076 sarcoma compared with ADM (Table 5). Two to four mice of six were cured of colon adenocarcinoma 38 when given 35 to 71 μmol/kg, and at those doses 85 to 97% tumor growth inhibition was observed in tumor-bearing mice. When ME2303 was given i.v. at a dose of 35 μmol/kg on Days 1, 8, 15, and 22 to mice bearing Lewis lung carcinoma, cure resulted in all mice, whereas ADM at the same schedule produced no cured mouse at doses of 8.9 to 34 μmol/kg. The drug given i.v. also induced remarkable growth inhibition against B16 melanoma, and 52 to 92% inhibition was observed at 18 to 71 μmol/kg. ME2303 was also effective against M5076 sarcoma, and at doses of 35 to 100 μmol/kg, more than 90% growth inhibition was obtained.

Growth-inhibitory Effect of ME2303 on Sensitive and Multidrug-resistant Tumor Cell Lines. ADM and its derivatives have

Table 4 *Antitumor effect of ME2303 and ADM administered i.v. against P388 leukemia*

P388 leukemia cells (10^6/mouse) were implanted i.p. into $CD2F_1$ mice (5 mice/group) on Day 0, and the drugs were administered i.v. on Day 1 or Days 1, 5, or 9.

Drug	Dose (μmol/kg/injection)	MST[a] (days)	ILS (%)	No. of cured mice[b]	Body wt change[c] (g)
Control	0	10.8 ± 1.8[d]			+0.6
		Treatment schedule: Day 1 only			
ME2303	4.4	9.8 ± 0.8	−9		−0.4
	8.9	13.0 ± 2.5	20		−1.0
	18	15.8 ± 2.5	46		−1.0
	35	17.0 ± 2.1	57		−0.8
	71	23.0 ± 2.6	113	2/5	−0.6
	106	21.7 ± 0.6	101	2/5	−2.4
	142			3/5	−2.8
	213	18.0 ± 10.5	67	2/5	−4.2
	248	6.2 ± 0.4	−42		−5.2
ADM	3.4	11.2 ± 1.9	−4		+0.6
	6.9	13.2 ± 3.3	22		−0.2
	14	17.5 ± 3.1	62	1/5	−0.8
	28	21.5 ± 0.6	99	1/5	−1.6
	55	18.5 ± 9.7	71	1/5	−3.6
	83	6.0 ± 0.0	−44		−3.9
		Treatment schedule: Days 1, 5, and 9			
ME2303	2.2	9.8 ± 0.8	−9		+1.0
	4.4	11.4 ± 2.6	5		+0.9
	8.9	14.0 ± 2.3	30		0
	18	21.6 ± 4.3	100		+0.2
	35	28.3 ± 2.9	162	1/5	0
	53			4/5	0
	71			5/5	−0.8
	106	11.5 ± 3.1	6	1/5	−2.2
	142	9.0 ± 1.2	42	1/5	−3.2
ADM	1.7	11.0 ± 3.2	2		+0.4
	3.4	9.4 ± 0.5	−13		+1.4
	6.9	15.2 ± 3.3	41		0
	14	24.6 ± 4.3	128		−0.2
	28	13.0 ± 1.2	20		−1.6

[a] MST, mean survival time of deceased mice.
[b] Cure incidence was determined on Day 61.
[c] Difference in body weight (g) between Days 5 and 1.
[d] Mean ± SD. Cured mice were excluded from the calculations. When more than 50% of the mice were cured, the mean survival time was not calculated.

Table 5 *Effect of ME2303 on murine tumors*

Tumors were implanted as described in "Materials and Methods," and the drug was given i.v. on Days 1, 8, 15, and 22.

Tumor	Drug	Dose (μmol/kg/injection)	MST[a] (days)	ILS (%)	Tumor growth inhibition (%)	No. of cured mice[b]	Body wt change[c] (g)
Colon adenocarcinoma 26	Control	0	26.2 ± 9.0[d]				+0.4
	ME2303	8.9	32.1 ± 11.5	23	23[e]	1/10	−0.2
		18	38.2 ± 8.0	46	76		−0.3
		35			99	8/10	−0.3
		71			100	8/10	−3.3
		106	7.7 ± 0.5	−71		3/10	−2.7
	ADM	8.6	36.9 ± 8.8	40	45		−0.2
		17	48.1 ± 5.5	83	87		0
		34	21.0 ± 3.2	−19			−2.3
Colon adenocarcinoma 38	Control	0					0
	ME2303	8.9			39[f]		+0.3
		18			48		+0.2
		35			85	2/6	0
		71			97	4/6	+0.6
	Control	0					+0.9
	ADM	6.9			22	1/6	+0.7
		14			88	3/6	+0.1
		28				1/6	−0.7
Lewis lung carcinoma	Control	0	28.0 ± 3.4[e]				−0.2
	ME2303	8.9	34.6 ± 4.2	24	19[g]	1/6	−0.3
		18	44.3 ± 6.6	58	43		−0.3
		35			100	6/6	−0.5
		71			100	4/6	−0.3
		106	6.0 ± 0	−79		2/6	−4.2
	Control	0	29.8 ± 4.2				+0.9
	ADM	8.9	38.4 ± 5.7	29	37		+0.1
		17	42.2 ± 3.8	42	74		+0.1
		34	27.3 ± 9.2	−8			+0.1
B16 melanoma	Control	0	33.8 ± 9.3				−1.2
	ME2303	18	32.2 ± 10.2	−5	52[f]	1/6	−0.5
		35	41.7 ± 9.2	23	48		−0.5
		71	42.3 ± 20.2	25	92	2/6	−1.3
		106	14.0 ± 7.8	−59		3/6	−1.2
	Control	0	28.0 ± 6.0				+1.0
	ADM	8.6	28.6 ± 2.3	−12	59		+1.0
		17	37.8 ± 5.6	35	79		+1.0
		34	24.6 ± 3.1	2			+0.9
M5076 sarcoma	Control	0	40.9 ± 7.2				+1.3
	ME2303	8.9	54.3 ± 2.9	33	40[g]	2/6	+0.7
		18			59	4/6	0
		35			96	6/6	−0.5
		71			100	6/6	−0.5
		106			100	5/6	−0.3
	ADM	8.6	41.7 ± 7.9	2	16		−0.3
		17	46.2 ± 2.8	13	88		−0.8
		34	33.8 ± 12	−17	91		−1.5

[a] MST, mean survival time of deceased mice.
[b] Cure incidence was evaluated on Day 61.
[c] Difference in body weight (g) between Days 8 and 1.
[d] Mean ± SD. Survivors were excluded from the calculations of the mean and SD. When more than 50% of the mice were cured, the mean survival time was not calculated.
[e] Tumor growth inhibition on Day 29.
[f] Tumor growth inhibition on Day 27.
[g] Tumor growth inhibition on Day 28.

been shown to be ineffective against multidrug-resistant tumor cells. The effectiveness of ME2303 against various multidrug-resistant tumor cells was examined (Table 6). Human multidrug-resistant tumor cells K562/ADM, CEM/VLB$_{100}$, 2780AD, and KBC-4 showed 200-, 3.8-, 392-, and 65-fold resistance to ADM when the IC_{50} values of these tumor lines and the parental cells were compared (Table 6). These same cells showed only 2.8-, 0.9-, 22-, and 2.9-fold resistance to ME2303.

Murine multidrug-resistant tumor cells P388/ADM and P388/VCR were 11- and 1.3-fold more resistant to ME2303 than were the parental P388 cells, whereas they were 153- and 11-fold more resistant to ADM than the parental cells. These results clearly indicate that ME2303 had a greater effect on multidrug-resistant tumor cells than ADM.

Chemotherapeutic Effect of ME2303 in VCR- and ADM-resistant Tumor-bearing Mice. The *in vivo* effect of ME2303 against a murine drug-resistant tumor was examined (Tables 7 and 8). ME2303, given on Day 1 against parental P388 leukemia, showed almost equal activity to that obtained by repeated (Days 1, 5, and 9) administration (Table 3). Therefore, the therapeutic effect of ME2303 by single i.p. injection was examined against P388/VCR and P388/ADM.

Superior chemotherapeutic effects were obtained with ME2303 in P388/VCR-bearing mice (Table 7). VCR at 2.4 μmol/kg given i.p. on Day 1 showed 78% ILS in mice bearing P388 tumors; however, VCR at 1.4 to 5.4 μmol/kg and ADM at 8.6 to 35 μmol/kg given i.p. on Day 1 showed a marginal chemotherapeutic effect (maximum ILS = 8% and 24%, respectively) in mice bearing P388/VCR tumors. On the other

Table 6 *Growth-inhibitory effect of ME2303 and ADM in drug-resistant murine and human tumor lines*

Tumor cells (2 to 4 × 10^4) were seeded in plastic dishes containing 2 ml of growth medium, and graded concentrations of drugs were added as described in "Materials and Methods." After 72 h of continuous drug exposure, the tumor cells were counted.

Cell line	IC_{50} (nM)	
	ME2303	ADM
K562	11 ± 0.4[a]	27 ± 0.4
K562/ADM	31 ± 1.4 (2.8)[b]	5400 ± 2.0 (200)
CCRF-CEM	22 ± 2.1	17 ± 0.4
CEM/VLB_{100}	20 ± 1.1 (0.9)	65 ± 1.4 (3.8)
A2780	3.4 ± 0.2	2.4 ± 0.2
2780AD	75 ± 3.8 (22)	940 ± 44 (392)
KB3-1	18 ± 0.0	8.8 ± 0.7
KBC-4	52 ± 3.7 (2.9)	570 ± 0.1 (65)
P388	13 ± 0.7	15 ± 0.2
P388/ADM	140 ± 23 (11)	2300 ± 3.8 (153)
P388/VCR	17 ± 1.4 (1.3)	170 ± 7.0 (11)

[a] Mean ± SD of three determinations.
[b] Numbers in parentheses, degree (x-fold) of resistance as compared with parent cells.

hand, ME2303 given on the same schedule resulted in 57 to 96% ILS at 18 to 71 μmol/kg, and two mice of six were cured with a 71-μmol/kg dose. Chemotherapeutic effects were obtained with ME2303 in P388/ADM tumor-bearing mice (Table 8). ADM doses of 12 to 34 μmol/kg showed no chemotherapeutic effect in mice bearing P388/ADM tumors (Table 8). ME2303 given on the same schedule resulted in about 30% ILS at the dose of 36 to 50 μmol/kg. Although these survival advantages were smaller than those conferred by ME2303 at similar doses in the parental P388 leukemia-bearing mice, ME2303 could be effective against pleiotropic drug-resistant tumor cells *in vivo*, especially against P388/VCR tumor-bearing mice.

DISCUSSION

ME2303 showed significant antitumor activity against various murine tumor models over wide dosage ranges. Another interesting characteristic of ME2303 is its effects on multidrug-resistant tumor cells. It is almost equally effective against multidrug-resistant human tumor cell lines and their parental cell lines *in vitro*. In animal experiments using P388/VCR and P388/ADM, the compound also showed good therapeutic effects. P388/VCR was weakly resistant to ADM *in vitro* (index of resistance was 11-fold; Table 6) but not resistant to ME2303. A prominent chemotherapeutic effect of ME2303 was observed *in vivo* against P388/VCR (Table 7). P388/ADM was highly resistant to ADM *in vitro* (index of resistance was 153-fold; Table 6) and showed 11-fold resistance to ME2303. ADM showed no therapeutic effect against P388/ADM *in vivo*, and ME2303 showed a partial but significant effect against P388/ADM *in vivo* (Table 8). These observations suggest that the drug-resistant index *in vitro* might correlate with a chemotherapeutic effect *in vivo* and might explain the better therapeutic effect of ME2303 against P388/VCR tumor-bearing mice *in vivo* than that against P388/ADM cells.

We have previously reported that combination therapy of VCR and verapamil rendered P388 leukemia-bearing mice tumor free (15). Verapamil is known to increase the effectiveness of VCR and ADM against multidrug-resistant tumor cells (13). Therefore, a combination of VCR and verapamil should be effective against a small subpopulation of drug-resistant P388 that may exist in parental P388 cells. In this report, ME2303 alone was shown to be effective against drug-resistant human and murine tumor cells. This is supposed to be one of the major reasons that ME2303 treatment resulted in a high incidence of tumor-free survivors.

ME2303 has now entered clinical Phase I study in Japan. According to the impressive effects of this compound on various animal tumors and also on some multidrug-resistant tumors, this compound holds considerable interest for further evaluation.

Table 7 *Antitumor activity of ME2303 and ADM on P388/VCR*

P388/VCR or P388 cells (10^6/mouse) were implanted i.p. into $CD2F_1$ mice (6 mice/group) on Day 0, and drugs were administered i.p. on Day 1.

	Drug	Dose (μmol/kg)	MST[a] (days)	ILS (%)	No. of cured mice	Body wt change[b] (g)
P388/VCR	Control	0	13.4 ± 1.9[c]			+0.9
	ME2303	8.9	17.2 ± 3.6	28	1/6	+0.4
		13	19.5 ± 5.3	46		+0.2
		18	22.8 ± 6.8	70	1/6	+0.2
		25	24.4 ± 6.5	82	1/6	−0.8
		35	22.4 ± 1.9	67	1/6	−1.7
		50	26.2 ± 2.5	96	1/6	−3.7
		71	21.0 ± 9.5	57	2/6	−4.5
	VCR	1.4	13.3 ± 2.1	−1		−1.6
		2.0	12.2 ± 0.8	−9		−2.5
		2.7	13.7 ± 2.6	2		−4.0
		3.8	14.5 ± 2.1	8		−3.2
		5.4	10.8 ± 3.7	−20		−2.4
	ADM	8.6	13.3 ± 6.1	−8		−0.2
		12	13.8 ± 2.3	2		−0.4
		17	16.7 ± 2.6	24		−1.5
		24	16.2 ± 4.3	20		−1.5
		35	16.0 ± 2.8	19		−2.6
P388	Control	0	10.4 ± 0.5			ND
	VCR	1.8	17.6 ± 1.1	69		ND
		2.4	18.5 ± 1.5	78		ND

[a] MST, mean survival time; ND, not determined.
[b] Difference in body weight (g) between Days 5 and 1.
[c] Mean ± SD. Survivors on Day 31 were excluded from the calculations of the mean and SD.

Table 8 *Antitumor activity of ME2303 and ADM on P388/ADM*

P388/ADM cells (10^6/mouse) were implanted i.p. into $CD2F_1$ mice (6 mice/group) on Day 0, and drugs were administered i.p. on Day 1.

Drug	Dose (μmol/kg)	MST[a] (days)	ILS (%)	Body wt change[b] (g)
Control	0	10.5 ± 1.4[c]		+1.0
ME2303	8.9	12.0 ± 1.6	14	0
	13	11.2 ± 1.0	6	−0.2
	18	12.5 ± 1.8	19	−1.4
	25	12.7 ± 3.1	20	−2.5
	36	13.5 ± 1.4	28	−2.5
	50	14.2 ± 1.9	34	−3.7
	71	9.5 ± 6.6	−10	−3.7
ADM	12	10.2 ± 0.4	−4	+0.1
	17	10.3 ± 1.9	−2	−2.7
	24	10.0 ± 1.3	−5	−1.2
	34	7.2 ± 3.6	−32	−2.2

[a] MST, mean survival time of deceased mice.
[b] Difference in body weight (g) between Days 5 and 1.
[c] Mean ± SD.

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