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Antitumor Activity of Mitoxantrone Against Murine Experimental Tumors: Comparative Analysis Against Various Antitumor Antibiotics

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Summary. *1,4-Dihydroxy-5,8-bis{{[2-[(2-hydroxyethyl)amino]ethyl}amino}-9,10-anthracenedione dihydrochloride (mitoxantrone) was tested for antitumor activity against experimental tumors in mice and the results were compared with those of seven antitumor antibiotics: adriamycin (ADM), daunomycin (DM), aclarubicin, mitomycin C (MMC), bleomycin, neocarzinostatin, and chromomycin A_3. The drugs were given IP or IV, in general on days 1, 5, and 9 following tumor inoculation. Mitoxantrone given IP at the optimal dose (1.6 mg/kg/day; as a free base) produced a statistically significant number of 60-day survivors (curative effect) in mice with IP implanted L1210 leukemia. The curative effect was not observed with any of the other antibiotics. In the case of IV implanted L1210 leukemia, there was an increase in lifespan (ILS) by more than 100% in the mice following IV treatment with mitoxantrone or DM. In IP implanted P388 leukemia, the curative effect was elicited by IP treatment with mitoxantrone or MMC. In IP implanted B16 melanoma, both the curative effect and a more than 100% ILS in mice that did die were produced by IP treatment with mitoxantrone or ADM. In SC implanted Lewis lung carcinoma, mitoxantrone and ADM administered IV also showed effective antitumor activities and produced a 60% and a 45% ILS, respectively. In conclusion, mitoxantrone and ADM had a wider spectrum of antitumor activity against mouse tumors, including two leukemias and two solid tumors, than did the other drugs; however, mitoxantrone elicited higher antitumor effects than ADM on mouse leukemias, especially on L1210 leukemias. Moreover, mitoxantrone possessed much higher therapeutic indices than ADM against IP implanted P388 (optimal dose/ILS_{40}; > 128 versus 15.2) and L1210 (optimal dose/ILS_{25}; 72.7 versus 4.8) leukemias. In addition, mitoxantrone showed moderate activity against DM-resistant L1210 leukemia.*

Introduction

With the object of finding a new compound possessing equal or greater antitumor activity but less cardiotoxicity than adriamycin (ADM), a series of bis (substituted aminoalkylamino) anthraquinones were synthesized and examined for therapeutic and toxicologic effects [8, 10, 11]. Of this series, 1,4-dihydroxy-5,8-bis{{[2-[(2-hydroxyethyl)amino]ethyl}amino}-9,10-anthracenedione dihydrochloride (mitoxantrone) was selected as one of the most reasonable candidates for clinical use, and the phase I studies of this drug were recently concluded [1, 9].

The purpose of this report is to describe the results of comparing the antitumor efficacy of mitoxantrone against experimental tumors in mice with that of various antitumor antibiotics currently in clinical use.

Materials and Methods

Drugs. Mitoxantrone, aclarubicin, bleomycin, neocarzinostatin, and chromomycin A_3 were kindly supplied by Lederle (Japan), Ltd, Tokyo, Japan; Sanraku Ocean Co., Ltd., Fujisawa, Japan; Nippon Kayaku Co., Ltd, Tokyo, Japan; Kayaku Antibiotics Research Co., Ltd, Tokyo, Japan; and Takeda Chemical Ind., Osaka, Japan, respectively. Adriamycin and mitomycin C (MMC) were purchased from Kyowa Hakko Kogyo Co., Ltd, Tokyo, Japan, and daunomycin (DM) from Meiji Seika Co., Ltd, Tokyo, Japan.

Drugs were dissolved in physiological saline. Solutions were freshly prepared and administered IP or IV to tumor-bearing mice in a volume of 0.01 ml/g body weight on days 1, 5, and 9, unless

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otherwise specified. The therapeutic doses employed for each drug were determined, in general, on the basis of the LD_{10} dose (dose required to kill 10% of non-tumor-bearing BDF_1 mice) obtained by us or by others [6] and the drug solutions were generally diluted by a factor of 2.

Animals and Tumors. Adult BDF_1 mice of either sex, weighing 20–26 g, were used for B16 melanoma, Lewis lung carcinoma, L1210 leukemia, and P388 leukemia in one experiment. Adult female CDF_1 mice weighing 18–23 g were used for P388 leukemia. L1210 and P388 leukemias were maintained by serial IP passage in female BDF_1 and CDF_1 mice, respectively. A DM-resistant subline of L1210 leukemia had been established in our laboratory and was maintained in female BDF_1 mice routinely receiving three injections of DM (1 mg/kg/day) on days 1, 3, and 5 after tumor inoculation. B16 melanoma and Lewis lung carcinoma were maintained SC in syngeneic adult male C57BL/6 mice. All the mice except CDF_1 mice (which were purchased from Charles River Japan, Inc., Kanagawa, Japan) and all the tumors were obtained from Drug Research and Development (DR & D), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.

Standardized protocols of the DR & D Program, NCI [4], with minor modifications [3], were followed for serial passage of the tumors and for implantation of tumors into BDF_1 or CDF_1 mice. The parent and DM-resistant sublines of L1210 leukemias were implanted IP or IV at 10^5 cells/mouse and P388 leukemia was implanted IP at 10^6 cells/mouse on day 0. B16 melanoma was implanted IP as 0.5 ml of a 1/9 (w/v) tumor brei and Lewis lung carcinoma was implanted SC (5×10^5 viable cells) on day 0.

Evaluation of Antitumor Activity. Antitumor activity of the drugs against all the tumors was assessed from two parameters: (a) the mean survival time of the drug-treated mice excluding long-term survivors versus saline-treated controls, expressed as percentage increase in mean lifespan (%ILS = T/C%−100); and (b) the incidence of long-term (60-day) survivors. The criteria of effective antitumor activity were the same as those employed for the 'DCT panel of Antitumor Screens' [5]. Statistical analysis was carried out according to Fisher's exact test for 60-day survival incidence, and chemotherapy, producing a significant number of survivors compared with that (none) of controls for each tumor, was evaluated as curative.

Results

Antitumor Activity of Drugs Against L1210 Leukemias

As shown in Table 1, mitoxantrone produced a significant number of 60-day survivors as well as a 153% ILS in ultimately deceased mice when used to treat IP implanted L1210 leukemia. Adriamycin produced a few (but not a significant number of) survivors, but the ILS of deceased mice was only 48%. The antitumor activity of the other drugs caused less than 100% ILS and no long-term survivors.

With IV implanted L1210 leukemia, more than 100% ILS was seen only in the group treated with mitoxantrone or DM (Table 2).

Antitumor Activity of Drugs Against P388 Leukemia

As shown in Table 3, all the mice that received IP implants of P388 leukemia survived for 60 days after tumor implantation when treated with mitoxantrone and MMC. Adriamycin also showed a high antitumor effect, but the difference in survival incidence against controls was not significant statistically. No survivors were observed in the groups treated with the other drugs.

Antitumor Activity of Drugs Against B16 Melanoma

Against IP implanted B16 melanoma, mitoxantrone and ADM produced significant numbers of 60-day

Table 1. Antitumor activity of mitoxantrone and seven antitumor antibiotics against L1210 leukemia (IP–IP)

Drug	Dose range (mg/kg/day)	Optimal dose (mg/kg/day)	ILS[a, b] (%)	60-day survival[d]
Mitoxantrone	3.2– 0.4	1.6	153	4/6*
Adriamycin	10 – 1.25	5	48 (214)[c]	2/6
Daunomycin	3 – 0.75	3	41	0/8
Aclarubicin	8 – 2	8	46	0/8
Mitomycin C	4 – 1	4	71	0/8
Bleomycin	74 –18.5	74	2	0/8
Neocarzinostatin	1.8– 0.45	0.9	39	0/8
Chromomycin A_3	0.8– 0.2	0.4	39	0/8

[a] Mean survival of saline-treated controls was 9.3 days
[b] ILS with the optimal dose
[c] Including the survivors as they survived for 60 days
[d] No. of 60-day survivors/total with the optimal dose
* $P < 0.05$

Table 2. Antitumor activity of mitoxantrone and seven antitumor antibiotics against L1210 leukemia (IV–IV)

Drug	Dose range (mg/kg/day)	Optimal dose (mg/kg/day)	ILS[a] (%)	60-day survival
Mitoxantrone	3.2– 0.4	3.2	112	0/8
Adriamycin	5.8– 0.7	5.8	41	0/8
Daunomycin	13 – 3.2	13	147	0/8
Aclarubicin	8 – 2	8	38	0/8
Mitomycin C	4.1– 1	4.1	41	0/8
Bleomycin	80 –20	20	–2	0/8
Neocarzinostatin	1.8– 0.45	0.9	88	0/8
Chromomycin A_3	1.3– 0.3	1.3	45	0/8

[a] Mean survival of saline-treated controls was 6.6 days

Table 3. Antitumor activity of mitoxantrone and seven antitumor antibiotics against P388 leukemia (IP–IP)

Drug	Dose range (mg/kg/day)	Optimal dose (mg/kg/day)	ILS[a] (%)	60-day survival
Mitoxantrone	3.2– 0.4	1.6	–	5/5*
Adriamycin	10 – 1.25	5	172	3/5**
Daunomycin	3 – 0.75	3	104	0/5
Aclarubicin	8 – 2	8	67	0/5
Mitomycin C	4 – 1	4	–	5/5*
Bleomycin	74 –18.5	37	19	0/5
Neocarzinostatin	1.8– 0.45	0.9	62	0/8
Chromomycin A_3	0.8– 0.2	0.4	93	0/5

[a] Mean survival of saline-treated controls was 9.6–11.4 days
* $P < 0.01$
** Not significant at $P < 0.05$

survivors and more than 100% ILS in ultimately deceased mice. On the other hand, the antitumor effects of the other drugs were less than 100% ILS, and there were no long-term survivors (Table 4).

Antitumor Activity of Drugs Against Lewis Lung Carcinoma

As shown in Table 5, SC implanted Lewis lung carcinoma was refractory to the drugs examined in the present study; however, more than 40% ILS was observed in the groups treated with mitoxantrone and ADM.

Summary of Antitumor Activity of Drugs

The maximum antitumor effects at the optimal doses of mitoxantrone and seven antitumor antibiotics in a veriety of tumor systems are summarized in Table 6. Mitoxantrone and ADM showed a wider spectrum of antitumor activities against mouse tumors, including two leukemias and two solid tumors, than did the other drugs. Furthermore, mitoxantrone showed more pronounced antitumor effects than ADM on murine leukemias, especially on L1210 leukemias implanted IP and IV. The superiority of mitoxantrone over ADM in antitumor activity against murine leukemias was further confirmed in the experiments in which the therapeutic indices of both drugs were determined with P388 leukemia and L1210 leukemias (Table 7). Mitoxantrone had higher therapeutic indices than ADM against IP implanted P388 and L1210 leukemias.

Antitumor Activity of Mitoxantrone and ADM Against DM-Resistant L1210 Leukemia

Antitumor activity of mitoxantrone against a DM-resistant subline of L1210 leukemia was examined to determine whether there was any evidence of cross-resistance between the anthracyclines and

Table 4. Antitumor activity of mitoxantrone and seven antitumor antibiotics against B16 melanoma (IP–IP)

Drug	Dose range (mg/kg/day)	Optimal dose (mg/kg/day)	ILS[b] (%)	60-day survival
Mitoxantrone	3.2– 0.4	3.2	121	5/8*
Adriamycin	10 – 1.25	2.5	138	6/8**
Daunomycin[a]	9 – 1.5	1.5	35	0/8
Aclarubicin[a]	30 – 5	20	13	0/8
Mitomycin C[a]	15 – 5	10	79	0/8
Bleomycin	80 –20	40	13	0/8
Neocarzinostatin	1.8– 0.45	0.45	41	0/8
Chromomycin A_3[a]	1.0– 0.2	0.4	22	0/8

[a] Administered on day 1 only
[b] Mean survival of saline-treated controls was 18.9–23.6 days
* $P < 0.02$
** $P < 0.01$

Table 5. Antitumor activity of mitoxantrone and seven antitumor antibiotics against Lewis lung carcinoma (SC–IV)

Drug	Dose range (mg/kg/day)	Optimal dose (mg/kg/day)	ILS[a] (%)	60-day survival
Mitoxantrone	3.2– 0.4	3.2	60	0/8
Adriamycin	7.5– 1.3	5	45	0/8
Daunomycin	13 – 3.25	3.25	9	0/8
Aclarubicin	8 – 2	2	– 3	0/8
Mitomycin C	4.1– 1	2	3	0/8
Bleomycin	100 –25	50	– 3	0/8
Neocarzinostatin	1.8– 0.45	0.9	31	0/8
Chromomycin A_3	1.3– 0.32	0.32	22	0/8

[a] Mean survival of saline-treated controls was 22.1–31.9 days

Table 6. Antitumor activity of mitoxantrone and seven antitumor antibiotics against four murine tumors[a]

Drug	L1210 leukemia		P388 leukemia	B16 melanoma	Lewis lung carcinoma
	(IP–IP)	(IV–IV)	(IP–IP)	(IP–IP)	(SC–IV)
Mitoxantrone	++++	+++	++++	++++	+
Adriamycin	+(+++)[b]	+	+++	++++	+
Daunomycin	+	+++	+++	+	–
Aclarubicin	+	+	++	–	–
Mitomycin C	++	+	++++	++	–
Bleomycin	–	–	–	–	–
Neocarzinostatin	+	++	++	+	–
Chromomycin A_3	+	+	+++	–	–

[a] Antitumor activities of each drug were graded as follows:

Criteria	L1210 leukemia	P388 leukemia	B16 melanoma	Lewis lung carcinoma
++++	Curable	Curable	Curable	Curable
+++	≧ 100% ILS	≧ 80	≧ 100	≧ 160
++	50–99	40–79	50–99	80–159
+	25–49	20–39	25–49	40–79
–	< 25	< 20	< 25	< 40

[b] Including the 60-day survivors as they survived for 60 days

Table 7. Comparison of therapeutic indices of mitoxantrone and adriamycin in murine leukemia systems

System	Drug	Dose range[b] (mg/kg/day)	Optimal dose (mg/kg/day)	$ILS_{40\text{ or }25}$[c]	Therapeutic index[d]
P388[a] (IP–IP)	Mitoxantrone	3.2–0.025	3.2	< 0.025	> 128
	Adriamycin	10 –0.31	5	0.33	15.2
L1210 (IP–IP)	Mitoxantrone	3.2–0.025	3.2	0.044	72.7
	Adriamycin	10 –0.31	5	1.05	4.8
L1210 (IV–IV)	Mitoxantrone	3.2–0.4	3.2	0.73	4.4
	Adriamycin	5.8–0.7	5.8	3.6	1.6

[a] BDF_1 mice were used as host animals
[b] Dilution factor was 2
[c] $ILS_{40\text{ or }25}$ = doses eliciting a 40% (P388) or a 25% (L1210) ILS, respectively
[c] Therapeutic index = optimal dose/ILS_{40} (P388) or optimal dose/ILS_{25} (L1210)

Table 8. Antitumor activity of mitoxantrone and adriamycin against daunomycin-resistant L1210 leukemia (IP–IP)

Drug	Dose (mg/kg/day)	Survival time (days)	ILS (%)	60-day survival
Mitoxantrone	1.6	15.6 ± 2.1[a]*	42	1/8
	0.8	15.6 ± 1.8*	42	0/8
Adriamycin	5	13.9 ± 1.6*	26	0/8
	2.5	12.4 ± 0.7**	13	0/8
Daunomycin	3	11.6 ± 0.9	5	0/8
	1.5	11.9 ± 1.0	8	0/8
Control	–	11.0 ± 1.1	–	0/8

[a] Mean ± SD
* $P < 0.001$
** $P < 0.01$ according to Student's *t*-test, as compared with saline-treated control

mitoxantrone. Adriamycin was used as a reference preparation. As shown in Table 8, both ADM and mitoxantrone produced significant increases in the survival time of leukemic mice; however, the degree of antitumor efficacy of ADM at the optimal dose was marginal (26% ILS), and it therefore appeared that DM-resistant L1210 leukemia essentially showed cross-resistance to ADM when this marginal effect was compared with the antitumor activity of ADM against parent L1210 leukemia (Table 1). Mitoxantrone, on the other hand, showed a moderate activity against this resistant tumor, and one of eight mice treated with this drug at the optimal dose survived for 60 days after tumor implantation. Thus, the cross-resistance to mitoxantrone of a DM-resistant subline of L1210 leukemia appeared to be partial.

Discussion

We examined antitumor efficacies of mitoxantrone against experimental tumors in mice, including two leukemias and two solid tumors, and the previously reported [8, 10, 11] highly significant activity against P388 and L1210 leukemias and against B16 melanoma was confirmed in the present study. This drug, when given IV, was also effective against SC implanted Lewis lung carcinoma (Tables 5 and 6). In addition, we extended the comparative study over seven antitumor antibiotics currently in clinical use, including ADM, and the superior antitumor activity of mitoxantrone over that of these other drugs was clearly shown in the present tumor systems (Table 6). Moreover, it was clearly shown that mitoxantrone possesses much higher therapeutic indices than ADM against IP implanted P388 and L1210 leukemias (Table 7).

In contrast to ADM, mitoxantrone showed a moderate activity against a DM-resistant subline of L1210 leukemia (Table 8). In addition, it was reported that an ADM-resistant subline of P388 leukemia showed cross-resistance to a number of DNA intercalators, mitotic spindle poisons, and protein synthesis inhibitors [7], but this resistant

subline did not show complete cross-resistance to mitoxantrone [8, 10]. These results, coupled with the significant antitumor activity against experimental tumors and the apparent lack of adverse cardiac effects in animals [2] and man [1, 9], may suggest that mitoxantrone is worth evaluating for clinical usefulness.

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