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A New Nitrosourea Derivative TA-077, 1-(2-Chloroethyl)-3-Isobutyl-3-(β-Maltosyl)-1-Nitrosourea

I. Comparative Study on Antitumor Activity

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Summary. *A new water-soluble nitrosourea derivative, 1-(2-chloroethyl)-3-isobutyl-3-(β-maltosyl)-1-nitrosourea (TA-077), was tested for antitumor activity against murine tumors and a human mammary carcinoma (MX-1) implanted in athymic mice, and the results were compared with those obtained with five other nitrosourea derivatives currently in clinical use: 1-(2-chloroethyl)-3-(methyl-α-D-glucopoyranos-6-yl)-1-nitrosourea (MCNU), 1-(2-chloroethyl)-3-(β-D-glucopyranosyl)-1-nitrosourea (GANU), 3-[(4-amino-2-methyl-5-pyrimidinyl)methyl]-1-(2-chloroethyl)-1-nitrosourea hydrochloride (ACNU), chlorozotocin, and 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (Me-CCNU).*

The results indicated that TA-077 had a unique optimal treatment schedule different from other nitrosoureas. With daily IV treatments for 5 days, TA-077 showed the highest antitumor activity of all against the advanced Lewis lung carcinoma, defined by tumor weight and the survival of tumor-bearing mice. Furthermore, TA-077 given according to this treatment schedule was successful in inducing an apparent cure (complete regression and no recurrence) in all the athymic mice bearing MX-1, which the other five nitrosoureas could not. In addition, TA-077 possessed higher therapeutic indices (optimal dose/ILS_{25}) against L1210 and P388 leukemias than MCNU, which possessed the highest therapeutic index against L1210 leukemia among the five nitrosourea derivatives.

Introduction

Chloroethylnitrosourea derivatives are an important family of anticancer agents. The original lipophilic nitrosoureas, such as BCNU, CCNU, and Me-CCNU, have achieved considerable antitumor efficacy in a variety of experimental [14] and human [17, 20–22] malignancies, but the delayed and cumulative hematologic toxicity [17, 20–22] has limited clinical use of these drugs.

Since streptozotocin and chlorozotocin (CZT) were found to successfully reduce myelosuppressive activity while preserving antitumor activity in experimental systems [2, 15], a variety of water-soluble nitrosourea derivatives with or without sugar carriers have been developed in Japan [1, 8, 10, 16, 18, 19] with the aim of obtaining a better antitumor spectrum but with reduced hematologic toxicity. The current status of development of these nitrosoureas and their clinical studies in Japan have been described previously [11–13].

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A new nitrosourea derivative bearing aminomaltose, TA-077 (Fig. 1), has been developed in Japan. This compound has the unique characteristics of being a masked compound and of having an optimal treatment schedule of five daily doses rather than a single dose [1]. TA-077 was shown to change by maltase to an active metabolite TA-G, 1-(2-chloroethyl)-3-isobutyl-3-(β-D-glucopyranosyl)-1-nitrosourea, in the experimental system [3]. The purpose of this report is to describe the experimental results comparing the antitumor efficacy of TA-077 against murine tumors and a human mammary carcinoma (MX-1) with those of several nitrosourea derivatives currently in clinical use.

Materials and Methods

Drugs. CZT and Me-CCNU were kindly supplied by Drug Research and Development (DR & D), Division of Cancer Treatment (DCT), National Cancer Institute (NCI), Bethesda, Md.; ACNU by Sankyo Co., Ltd, Tokyo, Japan; GANU by Meiji Seika Co., Ltd, Tokyo, Japan; MCNU by Tokyo Tanabe Co., Ltd, Tokyo, Japan; and TA-077 by Tanabe Seiyaku Co., Ltd, Osaka, Japan. Their structural profiles are shown in Fig. 1.

All the drugs, except Me-CCNU, were dissolved in physiological saline. Me-CCNU was suspended in physiological saline with a few drops of Tween 80 (Tokyo Kasei Co., Ltd, Tokyo, Japan). Solutions or suspensions of all drugs were freshly prepared, diluted generally by a factor of 2, and administered IP or IV to tumor-bearing mice in a volume of 0.01 ml/g body weight at the indicated time either in a single injection or in five injections, one being given on each of 5 consecutive days.

Animals and Tumors. Adult male BDF_1 mice weighing 22–26 g, adult female BDF_1 mice weighing 20–24 g, and adult male nude mice with a BALB/C genetic background (athymic mice) and weighing about 25 g were used in these studies. L1210 and P388 murine leukemias were maintained by serial IP passage in female BDF_1 and CDF_1 mice, respectively. An ACNU-resistant subline of L1210 leukemia had been established in our laboratory and was maintained in female BDF_1 mice routinely receiving three injections of ACNU (10 mg/kg/day), on days 1, 3, and 5 after tumor inoculation. Murine solid tumors, B16 melanoma and Lewis lung carcinoma, were maintained SC in syngeneic adult male C57BL/6 mice. Human mammary carcinoma (MX-1) diagnosed histologically as an infiltrating duct cell carcinoma (medullary tubular carcinoma)

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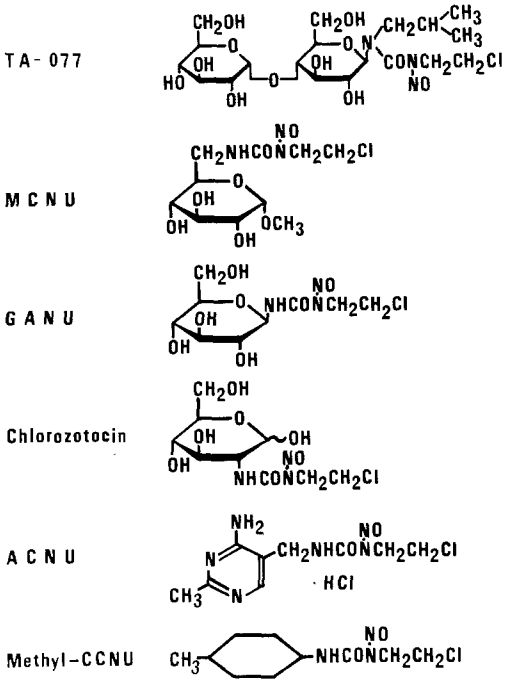

Fig. 1. Structure of TA-077, MCNU, GANU, CZT, Me-CCNU, and ACNU

was maintained SC in the athymic mice. BDF_1 and C57BL/6 mice and all the tumors were obtained from DR & D, DCT, NCI, Bethesda, Md. CDF_1 and the athymic mice were purchased from Charles River Japan, Inc., Kanagawa, Japan and from Clea Japan, Inc., Tokyo, Japan. The athymic mice which had been bred and maintained under specific-pathogen-free conditions were housed in the sterilized cages provided with sterilized food, tap water, bedding, and filter cap. All these cages were placed in a laminar-air-flow unit.

Standardized protocols of the DR & D Program, NCI [6], with minor modifications [5], were followed for serial passage of the murine tumors and for implantation of the murine tumors into BDF_1 or CDF_1 mice. The parent and ACNU-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. A fragment ($2 \times 2 \times 2$ mm) of MX-1 was implanted SC by a trocar into the flank of each athymic mouse on day 0.

Evaluation of Antitumor Activity. For the survival experiments, antitumor activity of the drugs against 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' [7]. 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) in controls for each tumor was evaluated as curative.

For the tumor-growth inhibition experiments, antitumor activity of the drugs against the advanced tumors was assessed from three parameters [4]: (a) the tumor-growth delay expressed as treated minus control (T−C, days), (b) the tumor-growth inhibition expressed as (1−T/C), and (c) the complete tumor-regression rate. Tumor-free survivors were excluded from the calculations in parameters (a) and (b). Tumor weights of Lewis lung carcinoma were calculated according to the formula: length × $(\text{width})^2 \times 0.5$ [6]. The tumor volumes of MX-1 were determined by three-dimensional measurements and the tumor volumes were transformed into relative values (V) by use of V = Vn/Vo, Vo being the volume at the initiation of treatment and Vn at any given day thereafter [9].

Results

Antitumor Activity of Drugs Against Early Murine Leukemias

As shown in Table 1, all five nitrosoureas were highly effective against the early L1210 leukemia, and almost all tumor-bearing mice given individual optimal doses survived for 60 days; however, according to the therapeutic index MCNU was the most potent of them (Expt 1). The antitumor activity of TA-077 against the early L1210 and P388 leukemias was therefore compared with that of MCNU in three different systems (Expts 2–4). Although both drugs were highly effective and curative at their optimal doses against the early L1210 and P388 leukemias in each system, TA-077 had higher therapeutic indices than MCNU in all cases examined.

Antitumor Activity of Drugs Against Advanced L1210 Leukemia and Cross-Resistance

Antitumor activities of six nitrosoureas against the advanced L1210 leukemias were examined by initiating treatment on days 4, 5, and 6 after tumor inoculation. As shown in Table 2, TA-077 was as effective as Me-CCNU, ACNU, and MCNU against the 4-day- and 5-day-old L1210 leukemias; however, the antitumor activity of TA-077 was inferior to that of any of these three nitrosoureas against the 6-day-old L1210 leukemia on the single-treatment schedule (Expt 1). The antitumor activity of CZT and GANU against the advanced L1210 leukemias was not so high as that of the other nitrosoureas (Expt 1).

Antitumor activity of five nitrosoureas against an ACNU-resistant subline of L1210 leukemia was examined to determine whether there was any evidence of difference in the mode of action of these drugs; however, this resistant subline showed complete cross-resistance to these sugar-carrying nitrosoureas (Expt 2).

Antitumor Activity of Drugs Against Murine Solid Tumors

As shown in Table 3, all nitrosoureas were highly effective and curative (except Me-CCNU) at their optimal doses (except GANU) against the early B16 melanoma (Expt 1); however, when treatments were initiated on day 4 the survivors could be seen only in the group treated with TA-077 or MCNU (Expt 2). Against the early Lewis lung carcinoma, TA-077, MCNU, Me-CCNU, and ACNU showed curative antitumor activity, whereas GANU and CZT did not show any effective antitumor activity (Expt 3). Similar results were obtained when the drugs were given IV on day 1 (data not shown).

Table 1. Comparison of therapeutic indices of six nitrosourea derivatives in murine leukemia systems

Expt no.	System[a]	Drug	Dose range[b] (mg/kg/day)	Optimal dose (mg/kg/day)	ILS_{25}[c] (mg/kg/day)	Therapeutic index[d]
1	L1210 (IP-IP, D1)	MCNU	80 −1.25	40 (5/5)[e]	1.25	32.0
		GANU	40 −0.625	10 (5/5)	0.8	12.5
		CZT	40 −0.625	40 (4/5)	2.4	16.7
		ACNU	80 −1.25	40 (5/5)	4.4	9.1
		Me-CCNU	80 −1.25	40 (5/5)	5.5	7.3
2	L1210 (IP-IP, D1−5)	TA-077	163 −1	65 (6/6)	1.6	40.6
		MCNU	15.6−0.1	12.5 (6/6)	0.52	24.0
3	P388 (IP-IP, D1−5)	TA-077	163 −1	32.5 (6/6)	<1.0	>32.5
		MCNU	15.6−0.1	6.3 (6/6)	0.4	15.8
4	L1210 (IV-IV, D1)	TA-077	127 −0.8	102 (6/6)	7.8	13.1
		MCNU	40 −0.25	32 (6/6)	4.2	7.6

[a] L1210 or P388 cells were inoculated IP or IV into five or six mice per group on day 0 and drugs were given IP or IV on day 1 (D1) or daily from days 1 to 5 (D1−5)
[b] Dilution factor was 2, including (Expt 1) or excluding (Expts 2−4) the highest dose used. (In the latter cases, the dilution factor between the highest and the next highest doses was 1.25)
[c] Dose eliciting a 25% increase in lifespan
[d] Optimal dose/ILS_{25}
[e] No. of 60-day survivors at the optimal dose/total number of mice in the group

Table 2. Antitumor activity of nitrosourea derivatives against advanced and ACNU-resistant L1210 leukemias

Expt no.	System[a]	Drug	Dose range (mg/kg)	Optimal dose (mg/kg)	ILS[b] (%)	60-day survivors
1	L1210 (IP-IP, D4)	TA-077	250−100	100	−	5/5*
		MCNU	50− 30	40, 30	−	5/5*
		GANU	30− 15	15	29	2/5**
		CZT	30− 15	30	4	1/5**
		Me-CCNU	50− 30	50, 40	−	5/5*
		ACNU	50− 30	40	−	5/5*
	L1210 (IP-IP, D5)	TA-077	250−100	100	167	4/5*
		MCNU	50− 30	50	81	4/5*
		GANU	30− 15	15	64	1/5**
		CZT	30− 15	30	47	0/5
		Me-CCNU	50− 30	50, 40	−	5/5*
		ACNU	50− 30	50	−	5/5*
	L1210 (IP-IP, D6)	TA-077	250−100	100	110	1/5**
		MCNU	50− 30	50	59	3/5**
		GANU	30− 15	30	29	1/5**
		CZT	30− 15	30	40	0/5
		Me-CCNU	50− 30	50	297	4/5*
		ACNU	50− 30	50	184	3/5**
2	L1210/ACNU (IP-IP, D1)	TA-077	160, 128	128	5	0/6
		MCNU	40, 20	40	20	0/5
		GANU	20, 10	10	22	0/5
		CZT	20, 10	20	17	0/5
		ACNU	40, 20	40	13	0/5

[a] Parent (L1210) and ACNU-resistant (L1210/ACNU) L1210 cells were inoculated IP into five or six mice per group on day 0 and drugs were given IP on day 1 (D1), day 4 (D4), day 5 (D5), or day 6 (D6) only
[b] Mean survival of saline-treated controls was 8.6−9.4 days (Expt 1) and 9.7 or 10.3 days (Expt 2), respectively. Only ILSs of deceased mice are presented
*$P < 0.05$
**Not significant at $P < 0.05$

Antitumor Activity of Drugs Against Advanced Lewis Lung Carcinoma

Antitumor activity of TA-077 against the advanced Lewis lung carcinoma implanted SC was compared with those of the other nitrosoureas. At first, the optimal treatment route and treatment schedule of TA-077 were examined in a 7-day-old Lewis lung carcinoma. As shown in Fig. 2, antitumor activity, defined by tumor weight and survival of the host, of TA-077 increased when administered IV rather than IP and when given by the multiple-treatment schedule rather than as a single treatment. As a result, in the groups studied the optimal mode

Table 3. Comparison of antitumor activity of six nitrosourea derivatives against B16 melanoma and Lewis lung carcinoma

Expt no.	System[a]	Drug	Dose range (mg/kg/day)	Optimal dose (mg/kg/day)	ILS[b] (%)	60-day survivors
1	B16 melanoma (IP-IP, D1)	TA-077	160–64	128	181	5/8*
		MCNU	45– 7.5	30	69	6/8*
		GANU	30– 5	20 (10)	– 44 (80)	5/8* (0/8)
		CZT	30– 5	20	56	5/8*
		Me-CCNU	60–10	40	119	3/8**
		ACNU	60–10	40	109	6/8*
2	B16 melanoma (IP-IP, D4)	TA-077[c]	130, 65	65	128	3/8**
		MCNU	40–10	40	86	2/6**
		GANU	20, 10	10	32	0/6
		CZT	20, 10	20	32	0/6
		Me-CCNU	40–10	40	67	0/6
		ACNU	40–10	40	81	0/6
3	Lewis lung carcinoma (SC-IP, D1)	TA-077	288–58	230	17	4/8*
		MCNU	80– 2.5	40	60	4/6*
		GANU	20– 1.25	5	21	0/6
		CZT	40– 1.25	20	32	0/6
		Me-CCNU	80– 2.5	40	53	5/6*
		ACNU	80– 2.5	40	36	4/6*

[a] B16 homogenate and Lewis lung carcinoma cells were implanted IP and SC, respectively, into six or eight mice per group on day 0, and drugs were given IP according to the schedules indicated

[b] Mean survival of saline-treated controls was 19.6 or 22.5 days (Expt 1), 20.9 or 21.0 days (Expt 2), and 20.9 or 25.6 days (Expt 3), respectively. Only ILSs of deceased mice are presented

[c] Administered daily from days 4 to 8

* $P < 0.05$

** Not significant at $P < 0.05$

Table 4. Comparison of antitumor activity of six nitrosourea derivatives against the advanced Lewis lung carcinoma implanted SC

Drug	Route and schedule	Dose range (mg/kg/day)	Optimal dose (mg/kg/day)	ILS[a] (%)	Tumor growth		
					T–C value[b] (days)	1–T/C[c] (%)	Complete regression rate[d]
TA-077	IV, qdx5	65–26	65	82	23.5	98	3/6 (1/6)[e]
MCNU	IP, 1 shot	100– 3.13	50	57	12.5	91	0/6
	IP, 1 shot	50–12.5	50	25	11.0	88	1/6 (0/6)
	IV, 1 shot	40, 30	30	65	11.0	96	0/6
GANU	IP, 1 shot	25– 0.8	12.5	14	0.5	44	0/6
	IP, 1 shot	50– 6.25	6.25	– 7	– 1.0	30	0/6
	IV, 1 shot	10, 7.5	7.5	23	2.0	38	0/6
CZT	IP, 1 shot	50– 1.6	12.5	16	–[f]	25	0/6
	IP, 1 shot	50– 6.25	25	– 3	2.5	46	0/6
	IV, 1 shot	20, 15	15	32	3.5	52	0/6
Me-CCNU	IP, 1 shot	50–12.5	50	52	15.5	97	1/6 (0/6)
ACNU	IP, 1 shot	50–12.5	50	31	11.0	90	0/6
	IP, 1 shot	50–30	40	44	8.5	86	0/6
	IV, 1 shot	50–30	30	57	9.5	93	0/6
	IV, 1 shot	40, 30	30	67	10.5	95	0/6

[a] Lewis lung carcinoma cells were implanted SC into six mice per group on day 0, and drugs were given IP or IV according to the indicated schedules when tumors reached a predetermined size (500 mg), usually day 7 or day 8. Mean survival of saline-treated controls was 21.8–29.5 days. Only ILSs of deceased mice are presented

[b] T–C value (days) = time required for the treatment group tumors (the mean value) to reach 1,000 mg minus the time required for the control-group tumors to grow to the same size, as reported elsewhere [4]

[c] 1–T/C (%) = percent degree of the tumor growth inhibition calculated by the mean tumor weight of the treated group excluding the completely tumor-regressed mice divided by the mean tumor weight of the control-group tumors (T/C). The maximum growth inhibition throughout the observation periods is shown [it was usually obtained 10–13 days after the (last) injections of drugs]

[d] Number of completely tumor-regressed mice/total number of mice in the group. The maximum rate throughout the observation periods is shown

[e] Number of tumor-free survivors at day 85/total number of mice in the group

[f] Mean tumor weight of this group did not reach 1,000 mg throughout the observation period after injection of the drug

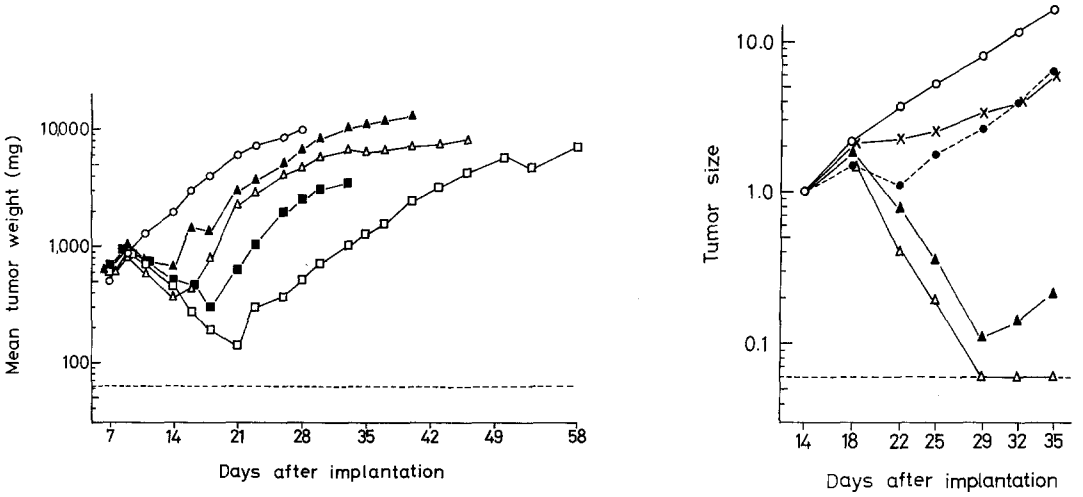

Fig. 2. Route and schedule-dependent antitumor activity of TA-077 against the advanced Lewis lung carcinoma. Lewis lung carcinoma cells were implanted SC into six mice per group on day 0 and the drug was given IP (▲, ■) or IV (△, □) on day 7 only (▲, △) or daily from days 7 to 11 (■, □). Tumor sizes were measured three times a week, in general, and the plots were terminated at the approximate median survival time of tumor-bearing mice in each group. – – – –, Limit of palpation of tumor (62.5 mg). The optimal doses, data for which are shown in this figure, and the dose ranges examined are: 160 (160, 128, and 64) mg/kg at IP, one shot (▲); 100 (100 and 50) mg/kg at IV, one shot (△); 81 (81, 65, and 32.5) mg/kg at IP, once daily × 5 (■); and 65 (65, 52, and 26) mg/kg at IV, once daily × 5 (□). Control animals (○) were treated IP with saline on day 7 only

Fig. 3. Antitumor activity of TA-077 against human breast cancer (MX-1) in athymic mice. A fragment of tumor was implanted SC into five athymic mice per group on day 0 and the drug was given IV on day 14 only (– – – –) or daily from days 14 to 18 (———). Tumor sizes (3 dimensions) were measured twice a week for 3 weeks after the initiation of treatments. – – – –, Limit of measurement of tumor (27 mm^3). Mean tumor size of each group at the initiation of treatment (day 14) ranged from 306 to 501 mm^3 (mean ± SD; 418 ± 72 mm^3) and each was normalized to 1.0. Groups were treated with 65 (△), 52 (▲), and 26 (×) mg/kg/day and groups treated with 100 mg/kg (●) or saline (○), respectively

of treatment for TA-077 was found to be five IV treatments distributed over 5 consecutive days. The antitumor activity of TA-077 that was observed at this optimal condition of treatment was tabulated and compared with those of the other drugs given IP or IV by a single injection, which appeared to be the optimal treatment schedule for the nitrosoureas [14]. As shown in Table 4, of all the drugs tested TA-077 produced the highest increase in the survival time of tumor-bearing mice as well as the most protracted tumor-growth delay, the highest degree of tumor-growth inhibition, and the highest number of mice with complete tumor regression.

Antitumor Activity of TA-077 Against Human Mammary Carcinoma (MX-1) in Athymic-Mice

Antitumor activity of TA-077 against a human tumor (MX-1) was examined by administering the drug IV because of the superior antitumor activity against the advanced Lewis lung carcinoma compared with IP administration (Fig. 2). As shown in Fig. 3, all the tumors of the group treated with the optimal dose (65 mg/kg/day) daily for 5 days regressed completely by 29 days after tumor inoculation (15 days after the initiation of treatment), and moreover, no recurrence of tumor was observed until 63 days after tumor inoculation (the observation period). Similarly, at the daily dose level of 52 mg/kg/day two of five tumors regressed completely and no recurrence of these tumors was observed. On the other hand, there were no tumor-free survivors in the group treated with a single injection containing the optimal dose (100 mg/kg) of TA-077; nor was any effective antitumor activity seen in the group that received a single injection of 50 mg TA-077/kg (data not shown).

Discussion

A single-high-dose therapy has been considered to be one of the best schedules for treatment of various experimental [14] and human [17, 20–22] malignancies with nitrosoureas. In the present study, however, a route- and schedule-dependent enhancement of antitumor activity was clearly shown for a new nitrosourea derivative, TA-077. Five IV injections of TA-077 over 5 consecutive days showed extensive antitumor activity against the advanced Lewis lung carcinoma (Fig. 2 and Table 4) and a human mammary carcinoma, MX-1 (Fig. 3), compared with TA-077 and the other nitrosoureas administered according to a single-treatment schedule. As shown previously [9], ACNU showed the highest antitumor activity against MX-1 among MCNU, GANU, CZT, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU), Me-CCNU, and ACNU; however, complete regression of the tumor was rarely seen and no apparently 'cured' mice were observed.

The mechanism(s) of increased antitumor activity of TA-077 when given by multiple daily doses is/are of interest. TA-077 is a masked compound and is inactive by itself against L1210, L5178Y, and HeLa cells in vitro [3]. Thus, pharmacokinetics of the active metabolite, TA-G, following the five IV administrations of TA-077 over 5 consecutive days needs to be studied in experimental systems. Furthermore, the distribution and activity of the activating enzyme, maltase, in tissues and organs of various animals, and the possibility of induction of the enzyme following multiple administration of the drug, clearly require clarification. Such investigations might yield valuable information that would allow us to establish the 'true' optimal treatment schedule if it is other than that described in the present study.

Of particular interest, on the other hand, was the hematologic toxicity of TA-077. In our preliminary experiment

(data not shown), a single IP dose of TA-077 at 160 mg/kg (LD_{10}) did cause delayed-type toxicity in the form of peripheral WBC counts essentially similar to those observed for Me-CCNU, MCNU, and ACNU, as shown previously [13]. However, our present concern is the hematologic toxicity of TA-077 with the five IV treatments over 5 days, TA-077 given according to this schedule showing far more effective antitumor activity than when given in a single treatment. Comparative toxicological studies, which include hematologic toxicity, are now in progress.

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