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Antitumor Activities of a Novel 9-Aminoanthracycline (SM-5887) against Mouse Experimental Tumors and Human Tumor Xenografts

Shinya Morisada, Yoshikazu Yanagi, Toshihiro Noguchi, Yasuo Kashiwazaki and Masaru Fukui

Research Laboratories, Sumitomo Pharmaceuticals Co., Ltd., 1-98, Kasugadenaka 3-Chome, Konohana-ku, Osaka 554

The antitumor effects of SM-5887, a totally synthetic 9-aminoanthracycline derivative, were evaluated in six murine experimental tumor systems (P388, Ehrlich carcinoma, sarcoma 180, Lewis lung carcinoma, B16 melanoma and colon 38) and nine human tumor-nude mouse systems (one breast cancer, two lung cancers and six gastric cancers). Characteristically SM-5887 showed excellent antitumor activities, superior to adriamycin (ADR), against human tumor xenografts, although its activities against murine experimental tumors were almost equal to those of ADR. When the human tumors were implanted sc in female athymic mice (BALB/c, *nu/nu*) and their volume reached 100–300 mm^3, SM-5887 and ADR were injected iv. All nine human tumors tested showed statistically significant responses to SM-5887, and 7 of them were strongly suppressed in their growth by SM-5887 so that minimum T/C values were less than 30% at the maximum tolerated dose (MTD, 25 mg/kg) with a single iv injection. Compared with ADR, SM-5887 was statistically more effective in five tumors (one breast, one lung and three gastric), equal in two tumors (two gastric), and less potent in two tumors (one lung and one gastric). In addition, the 10-day-interval repeated iv treatments with SM-5887 at the MTD (25 mg/kg) resulted in remarkably potent antitumor effects (including complete regression) against human gastric cancer, 4-1ST, implanted in nude mice without enhancement of toxic effects. SM-5887 was also effective against ip-inoculated P388 by oral administration as well as iv injection.

Key words: Anthracycline — SM-5887 — Antitumor activity

The anthracyclines, and in particular adriamycin (ADR), are widely used antitumor agents that have potent therapeutic activities against a variety of human cancers, and extensive investigations of their derivatives have been carried out in many laboratories in order to find new anthracyclines with higher antitumor activities and lower side effects.[1–6] Many compounds have been taken into clinical evaluation, but most of them are unlikely to have a clear advantage over ADR in terms of antitumor activities.[5] The compounds tested clinically so far, however, have been limited to those produced by fermentation or semisynthesis.[5]

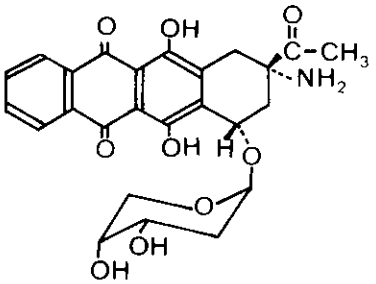
We undertook a different approach using totally synthetic anthracyclines to obtain compounds superior to ADR.[7] This enabled us to select compounds which possessed the amino group at the 9-position instead of the hydroxy group which invariably occurred in other anthracyclines,[6] and we found SM-5887, which is characterized by the 9-amino group and a simple sugar moiety different from daunosamine (Fig. 1).

It is very difficult to predict the clinical efficacy of antitumor agents, but the human tumor-nude mouse systems have been reported to be more useful for prediction than the mouse experimental tumor systems.[8, 9] In the present study, SM-5887 was shown to have excellent antitumor activities (superior to ADR) against human tumor xenografts, although its activities were almost equal to those of ADR against mouse experimental tumors. Since ADR is among the most useful drugs in cancer chemotherapy, the data suggest that SM-5887 is a promising antitumor agent worthy of clinical study.

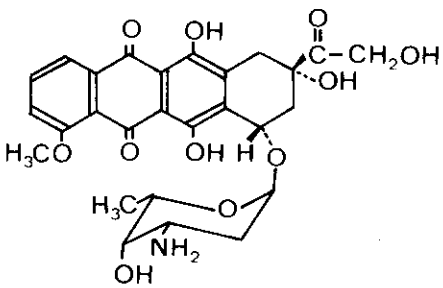
MATERIALS AND METHODS

Chemicals SM-5887 was prepared in Sumitomo Pharmaceuticals Co., Ltd. (Osaka),[7] and the purity was 94–95%. ADR was obtained from Kyowa Hakko Co., Ltd. (Tokyo). SM-5887 was dissolved in citrate-phosphate buffer containing 5% lactose (pH 3.8). ADR was dissolved in 0.85% NaCl solution. These drugs were administered to tumor-bearing mice at 0.1 ml/10 g of body weight.

Animals and tumors Male ICR/Jcl mice were purchased from Shizuoka Experimental Animals Corp. (Hamamatsu). Male DBA/2, C57BL/6, BALB/c×DBA/2 F1 (hereafter called CDF1) and C57BL/6×DBA/2 F1 (hereafter called BDF1) mice were obtained from Charles River Japan, Inc. (Kanagawa). P388 leukemia was maintained by serial intraperitoneal (ip) passage in DBA/2 mice. Sarcoma 180 and Ehrlich carcinoma were maintained by serial ip passage in ICR/Jcl mice. Lewis lung carcinoma was maintained intramuscularly (im) in C57BL/6 mice, while B16 melanoma and colon adenocarcinoma 38 were maintained subcutaneously (sc)



SM-5887



Adriamycin

Fig. 1. Structures of SM-5887 and adriamycin.

Table I. Characteristics of Human Tumor Xenografts

Tumor	Tissue of origin	Histopathologic characteristics
MX-1	Breast	Infiltrating duct carcinoma
LX-1	Lung	Small cell carcinoma
QG-56	Lung	Squamous cell carcinoma
SC-6	Stomach	Tubular adenocarcinoma, poorly differentiated type
SC-7	Stomach	Moderately differentiated tubular adenocarcinoma
SC-9	Stomach	Well differentiated tubular adenocarcinoma
St-4	Stomach	Poorly differentiated tubular adenocarcinoma
St-15	Stomach	Gelatinous adenocarcinoma
4-1ST	Stomach	Poorly differentiated adenocarcinoma

in C57BL/6 mice. Female athymic nude mice, BALB/c (*nu/nu*), were purchased from CLEA Japan Inc., Tokyo, and were housed under specific pathogen-free conditions. A breast cancer, MX-1, and lung cancers, LX-1 and QG-56, were supplied by the Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo. Gastric cancers, SC-6, SC-7, SC-9, St-4, St-15 and 4-1ST, were provided by the Central Institute for Experimental Animals, Kanagawa. These tumors were maintained by serial transplantation in nude mice. The characteristics of the human tumors used are listed in Table I.

Evaluation of antitumor activities against mouse experimental tumors P388 leukemia cells were implanted ip in CDF1 mice at 10^6 cells/mouse. Ehrlich carcinoma and sarcoma 180 were implanted im in ICR/Jcl mice at 3×10^6 cells/mouse, and 10^6 cells/mouse, respectively. Lewis lung carcinoma cells were implanted im in BDF1 mice at 10^6 cells/mouse. One-half ml of cell suspension of B16 melanoma (10% w/v) and colon adenocarcinoma 38 (14% w/v) was implanted sc in the flank of BDF1 mice. Each experimental group was composed of 6 mice. Drugs were administered iv or orally (po) 24 h after tumor implantation (day 1). Antitumor activity against P388 leukemia was determined by comparing the median survival time of the treated group with that of the control group and expressed as percentage of increased life span (ILS %). The growth of im-implanted Ehrlich carcinoma, sarcoma 180, and Lewis lung carcinoma was determined by weighing the tumor masses removed from the mice at 15, 13 and 12 days after tumor implantation, respectively. The growth of sc-implanted B16 melanoma and colon adenocarcinoma 38 was determined by measuring the length and width of the tumors with a sliding caliper at 16 days after tumor implantation. The estimated tumor volume (V) was expressed as $V=1/2\times\text{length (mm)}\times[\text{width (mm)}]^2$. Antitumor activities against solid tumors were determined by comparing the tumor weight or volume of the treated group (T) with that of the control group (C) and expressed as T/C values.

Evaluation of antitumor activities against human tumors The human tumor masses were excised and cut into about 3 mm × 3 mm pieces. Then, each fragment was implanted sc at a ventral site in a nude mouse with a trocar needle. When the tumor volume reached 100–300 mm^3, nude mice were separated into groups consisting of 6 mice after randomization and drugs were administered iv. The growth of tumors was measured twice a week, and the estimated tumor volume was calculated by the same methods used for the sc-implanted mouse tumors. The T/C% values were calculated by comparing the average tumor volume of the treated group with that of the control group each time that the tumors were measured, and the lowest value was expressed as minimum T/C% for each group. The statistical analysis was carried out by means of the Mann-Whitney U-test.

RESULTS

Antitumor activities against experimental mouse tumors The antitumor activities of SM-5887 against six mouse experimental tumors are compared with those of ADR in Table II. Drugs were administered iv once on day 1. The doses were 10 to 25 mg/kg for SM-5887 and 5 to 12.5 mg/kg for ADR.

Against P388 leukemia inoculated ip, SM-5887 showed the maximum ILS value of 122% at the dose of 25 mg/kg, and this activity was almost equal to that of ADR. Against solid Ehrlich carcinoma and sarcoma 180, SM-5887 showed potent activities, with minimum T/C values of 3% and 0%, respectively, at the dose of 20 mg/kg. These activities were superior to those of ADR, which showed a minimum T/C value of 39% against Ehrlich carcinoma and 20% against sarcoma 180. SM-5887 exhibited almost the same activities as those of ADR against Lewis lung carcinoma inoculated im and colon adenocarcinoma 38 inoculated sc. Against B16 melanoma inoculated sc, SM-5887 displayed a minimum T/C value of 31% at 25 mg/kg. This activity was less potent than that of ADR, which had a minimum T/C value of 5%.

Antitumor activities with oral administration The antitumor activities of SM-5887 administered po were evaluated in mice bearing P388 leukemia. Mice were

Table II. Antitumor Effects of SM-5887 and ADR on Mouse Experimental Tumors

Tumor	Tumor site		Dose (mg/kg)									
			SM-5887					ADR				
			25	20	16	12.5	10	12.5	10	8	6.3	5
Ascitic tumor												
P388 leukemia	ip	ILS%[a]	122	81	71			100	80	71		
Solid tumor												
Ehrlich carcinoma	im	T/C%[b]	toxic	3	3	14		toxic	39			61
Sarcoma 180	im	T/C%[b]		0	1	33	50	toxic	20	30	43	
Lewis lung carcinoma	im	T/C%[b]	toxic	36	48	65		38	54	76	69	75
B16 melanoma	sc	T/C%[c]	31	43	66	74	63	5	6	7	12	21
Colon adenocarcinoma 38	sc	T/C%[c]	17	27	9	22	40	toxic	5	26	7	23

Tumor cells were implanted into the indicated sites of mice on day 0, and drugs were administered iv once on day 1. Each group was composed of 6 mice.

a) Percent increase of life span.

b) Determined as ratio (%) of mean tumor weight of the treated group to that of the untreated one.

c) Determined as ratio (%) of mean estimated tumor volume of the treated group to that of the untreated one.

Table III. Antitumor Activities of SM-5887 Administered iv and po against P388 Leukemia

Drug	Route of administration	Dose (mg/kg)	Survival time (days)		% increase of life span
			Median	Range	
Untreated control			10.9	10–11	
SM-5887	iv	40	6.3	6–7	−43
		20	18.0	17–19	65
		10	14.4	14–15	32
		5	12.2	12–13	11
		2.5	11.2	11–12	2
SM-5887	po	70	20.3	18–24	86
		35	16.4	16–17	50
		17.5	14.3	13–15	31
		8.8	12.1	12–12	11
		4.4	11.1	10–12	1

P388 leukemia cells were implanted ip in CDF1 mice at 10^6 cells/mouse on day 0, and SM-5887 was administered iv or po once on day 1. Each group was composed of 6 mice.

Table IV. Antitumor Effects of SM-5887 and ADR against Human Breast and Lung Tumors Implanted into Nude Mice

Tumor	Drug	Dose (mg/kg)	Days[a]	Minimum T/C%[b]	P[c]
Breast					
MX-1	SM-5887	12.5	10	60 (7)	<0.05
		25		13 (7)	<0.001
	ADR	12.5		30 (17)	<0.001
Lung					
LX-1	SM-5887	12.5	10	63 (10)	<0.05
		25		25 (10)	<0.005
	ADR	12.5		44 (10)	<0.01
QG-56	SM-5887	25	10	53 (8)	<0.02
	ADR	12.5		28 (22)	<0.005

Tumor fragments were implanted sc into female BALB/c (*nu/nu*) mice. Drugs were injected iv when the tumor volume reached 100–300 mm^3. Each group was composed of 6 mice.
a) Days after tumor implantation when the treatment was started.
b) Minimum T/C% is the lowest value of T/C% for each group. The T/C% values were calculated by comparing the mean tumor volume of the treated group with that of the control group each time that the tumors were measured. Numbers in parentheses show the day of observation (after the start of treatment).
c) Analysis performed by means of the Mann-Whitney U-test, controls versus treated groups.

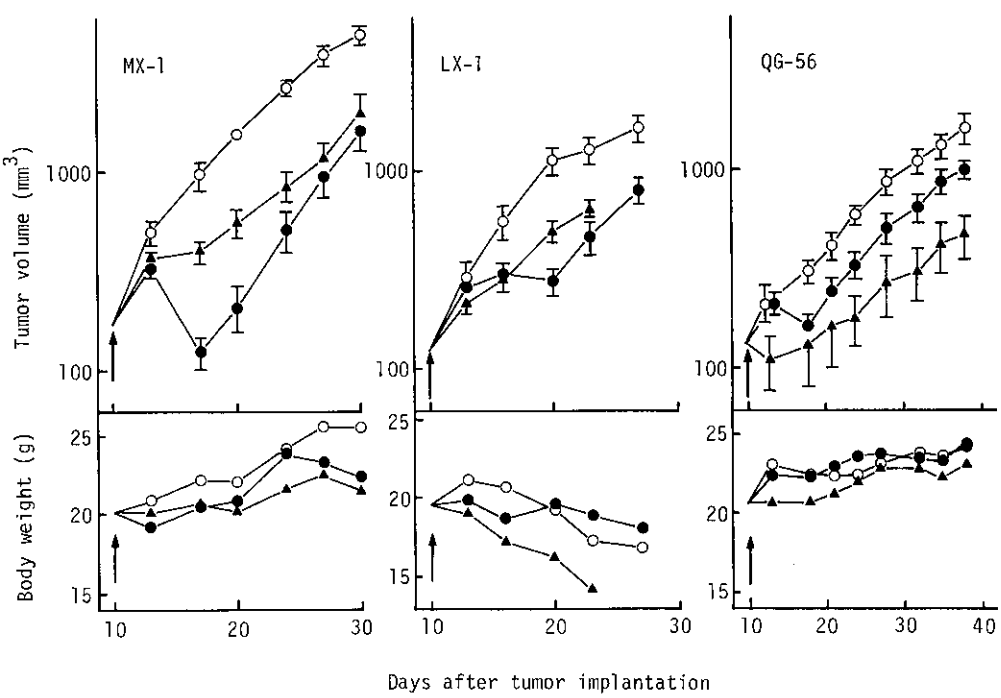


Fig. 2. Effects of SM-5887 and ADR on the growth of human tumor xenografts (MX-1, LX-1, and QG-56) in nude mice. Groups of 6 nude mice implanted with a tumor were untreated (○), given SM-5887 iv at a dose of 25 mg/kg (●), or ADR iv at a dose of 12.5 mg/kg (▲). Each value of the tumor volume represents the mean ±SE. Each value of the body weight represents the mean, and the coefficient of variation was less than 17% at each point. Arrows indicate administrations.

Table V. Antitumor Effects of SM-5887 and ADR against Human Gastric Tumors Implanted into Nude Mice

Tumor	Drug	Dose (mg/kg)	Days[a]	Minimum T/C%[b]	P[c]
Stomach					
SC-6	SM-5887	12.5	13	29 (7)	<0.02
		25		12 (10)	<0.001
	ADR	12.5		38 (17)	<0.005
SC-7	SM-5887	25	14	51 (20)	<0.01
	ADR	12.5		52 (17)	<0.005
SC-9	SM-5887	25	24	27 (14)	<0.01
	ADR	12.5		45 (14)	<0.05
St-4	SM-5887	12.5	9	46 (11)	<0.001
		25		23 (11)	<0.001
	ADR	12.5		16 (11)	<0.001
St-15	SM-5887	12.5	13	44 (31)	<0.02
		25		45 (28)	<0.01
	ADR	12.5		40 (28)	<0.001
4-1ST	SM-5887	12.5	10	12 (10)	<0.001
		25		4 (17)	<0.001
	ADR	12.5		26 (21)	<0.001

Tumor fragments were implanted sc into female BALB/c (*nu/nu*) mice. Drugs were injected iv when the tumor volume reached 100–300 mm^3. Each group was composed of 6 mice.
a) Days after tumor implantation when the treatment was started.
b) Minimum T/C% is the lowest value of T/C% for each group. The T/C% values were calculated by comparing the mean tumor volume of the treated group with that of the control group each time that the tumors were measured. Numbers in parentheses show the day of observation (after the start of treatment).
c) Analysis performed by means of the Mann-Whitney U-test, controls versus treated groups.

inoculated ip with P388 leukemia, and 24 h later SM-5887 was given po or iv. As shown in Tale III, po administration of SM-5887 was as effective as iv administration.

Antitumor activities against human tumors transplanted into nude mice The antitumor activities of SM-5887 against 9 human tumors (one breast cancer, two lung cancers and 6 gastric cancers) were compared with those of ADR. SM-5887 and ADR were administered iv once on the day when the tumor volume reached 100 to 300 mm^3. The doses of SM-5887 were 25 mg/kg (MTD) and 12.5 mg/kg (1/2MTD), and the dose of ADR was 12.5 mg/kg (MTD).

SM-5887 and ADR were active against one breast and two lung cancers tested (Table IV and Fig. 2). SM-5887 at the MTD showed potent antitumor activities, with a minimum T/C value of 13% against MX-1 ($P<0.001$), and tumor regression was seen 5 to 7 days after administration. ADR showed significant activities against MX-1, and a minimum T/C value of 30% was observed.

Against LX-1, SM-5887 showed a minimum T/C value of 25% ($P<0.005$) at MTD and ADR displayed a minimum T/C value of 44% ($P<0.01$). The LX-1 tumor caused severe body weight loss in the host mice. ADR accelerated the body weight loss in LX-1-bearing mice, but SM-5887 hardly affected it.

SM-5887 at the MTD showed statistically significant activity with a minimum T/C value of 53% ($P<0.02$) against the QG-56 tumor, but ADR activity with a minimum T/C value of 28% ($P<0.005$) was more potent than that of SM-5887.

All 6 human gastric cancers tested were susceptible to SM-5887 and ADR (Table V and Fig. 3). Among them, SC-6, SC-9, St-4 and 4-1ST evidenced a marked response to SM-5887 with minimum T/C values of less than 30% at MTD. In particular, SM-5887 caused an apparent regression of SC-6 and 4-1ST. SM-5887 showed statistically significant activities against SC-7 and St-15, but they were lower than those against the other gastric tumors. ADR had a potent activity against St-4, with a minimum T/C value of 16%, which was superior to that of SM-5887. ADR, on the other hand, manifested the same activities against SC-7 and St-15 and less potent activities against SC-6, SC-9 and 4-1ST compared with SM-5887.

In addition, the antitumor activities of SM-5887 given by multiple administrations were investigated. Nude mice bearing human gastric cancer, 4-1ST, were injected iv

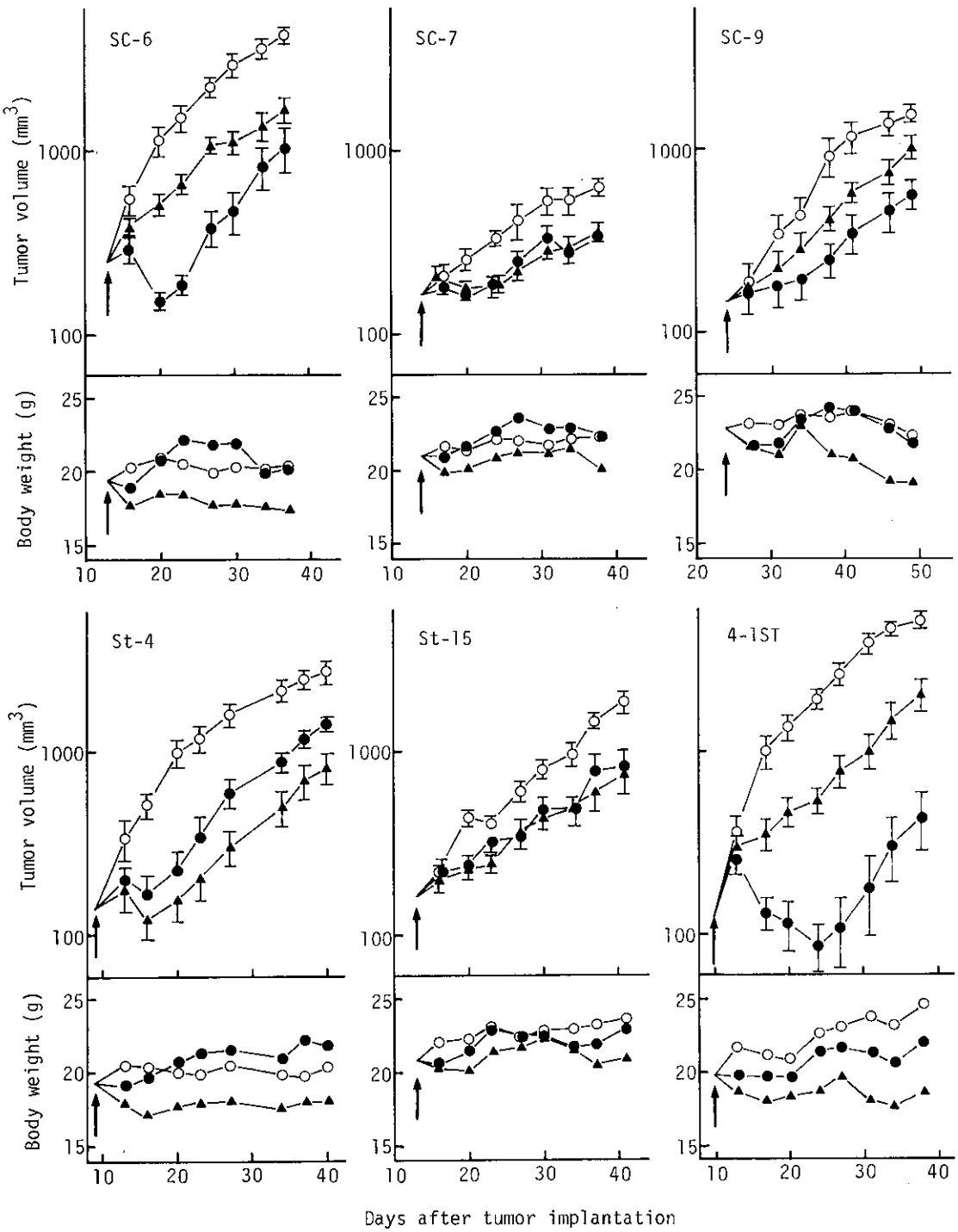


Fig. 3. Effects of SM-5887 and ADR on the growth of 6 gastric human tumor xenografts in nude mice. Groups of 6 nude mice implanted with a tumor were untreated (○), given SM-5887 iv at a dose of 25 mg/kg (●), or ADR iv at a dose of 12.5 mg/kg (▲). Each value of the tumor volume represents the mean±SE. Each value of the body weight represents the mean, and the coefficient of variation was less than 12% at each point. Arrows indicate administrations.

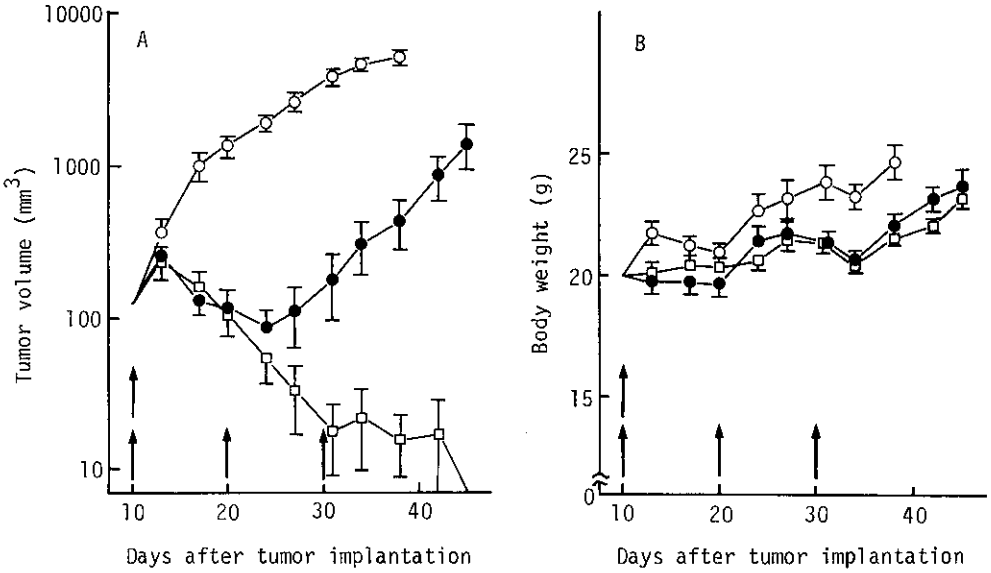


Fig. 4. Effects of multiple administrations of SM-5887 on the growth of human tumor xenograft 4-1ST (A) and the body weight change (B) in tumor-bearing nude mice. Groups of 6 nude mice implanted with tumor were untreated (○), given SM-5887 iv at a dose of 25 mg/kg with a single injection (●) or 3 injections every 10 days (□). Each point represents the mean±SE. Arrows indicate administrations.

with SM-5887 three times at 10-day intervals. Repeated administrations of SM-5887 exerted greater antitumor effects (including complete tumor regression) on 4-1ST than a single injection (Fig. 4).

DISCUSSION

The antitumor activities of SM-5887 were evaluated using six murine experimental tumor systems and nine human tumor-nude mouse systems, and were compared with those of ADR.

It is notable that SM-5887 showed excellent antitumor activities (superior to ADR) against human tumor xenografts. All nine human tumors tested, six gastric, two lung and one breast, exhibited statistically significant responses to SM-5887 ($P<0.01$), and seven of them, except one gastric and one lung, were strongly suppressed in their growth by SM-5887 so that the minimum T/C values were less than 30% . Compared with ADR, SM-5887 was statistically more effective in 5 of nine tumors, equal in 2 tumors, and less potent in 2 tumors ($P<0.05$). In this case, SM-5887- and ADR-treated tumor volumes were compared each time that the tumors were measured, and a significant difference between two groups required statistical significance ($P<0.05$) for at least one point.

SM-5887 was almost equipotent to ADR against the murine experimental tumors. The conventional murine tumor systems have contributed greatly to the development of new antitumor agents, but they are not necessarily useful to predict clinical efficacy, because many active compounds found in these systems have been clinically inactive. On the other hand, the human tumor-nude mouse systems have been expected to be more useful for the identification of new anticancer agents of clinical interest, and in particular, it has been reported that the effects of ADR against several human tumor xenografts reflected the clinical response rate very closely.[8,9] ADR is one of the most potent antitumor agents currently available, but its activities are not necessarily sufficient in terms of curative therapy of cancer patients. More active antitumor agents, therefore, are still needed. Since SM-5887 exhibited excellent antitumor activities (superior to ADR) in human tumor-nude mouse systems, it could be a promising candidate for clinical studies.

The MTD of SM-5887 could be repeatedly administered by single iv injection to mice at 10-day intervals, and showed remarkably potent activities (including complete regression) against human gastric cancer, 4-1ST, implanted in nude mice. On the other hand, the MTD of ADR could not be repeatedly administered, and even if ADR was injected at feasible dosages at 10-day intervals, it did not result in enhancement of antitumor activities (the data are not shown). These data also indicate the excellent properties of SM-5887, different from those of ADR, as an antitumor agent.

SM-5887 was effective against ip-inoculated P388 mouse leukemia cells by po administration as well as iv injection. Generally, the anthracyclines are known to be hardly absorbed from the gastrointestinal tract, although it has been reported that a few compounds were effective by po administration.[10-12] Effectiveness by the oral route is one of the characteristics of SM-5887.

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