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Antitumor Activity of Paclitaxel Against Human Breast Carcinoma Xenografts Serially Transplanted into Nude Mice

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Background: Paclitaxel (BMS-181339: Taxol) is a promising agent against previously treated breast cancer. The antitumor activity of paclitaxel was evaluated using five human breast carcinoma xenografts in nude mice.
Methods: Paclitaxel at 20 mg/kg dissolved in 0.2 ml ethanol/cremophor EL solution was administered intraperitoneally daily for 5 days.
Results: Paclitaxel showed significant antitumor activity against MCF-7 and MX-1, but only limited activity against the other three xenografts (R-27, Br-10, and T-61), suggesting its substantially different antitumor spectrum from conventional antibreast cancer drugs. The different sensitivity of xenografts to paclitaxel was successfully reproduced in vitro using the MTT assay, when the cutoff concentration of paclitaxel was 20 μg/ml.
Conclusion: Since no significant differences were observed in the pharmacokinetics of paclitaxel in sensitive and resistant tumor cell lines, the efficacy of this agent seemed to depend on the sensitivity of tumor cells rather than the intratumoral concentration of agent. J. Surg. Oncol. 64: 115–121.

KEY WORDS: **paclitaxel; nude mouse; breast neoplasms; pharmacokinetics**

INTRODUCTION

Paclitaxel (BMS-181339: Taxol) is a recently introduced antitumor agent initially extracted from the bark of the Pacific yew (*Taxus brevifolia*) [1]. This agent has demonstrated potent antitumor activity against many tumor models, including murine leukemias, murine solid tumors, and human tumor xenografts [2–5]. The mode of action of paclitaxel is a unique stabilizing effect on microtubules, resulting in inhibition of the dynamic reorganization of the microtubule network [6–10]. Potent antitumor activity of paclitaxel on breast [11] and ovarian [12] carcinomas has been reported in the United States, and a Phase II study on ovarian, breast, nonsmall cell lung, and gastric carcinomas has been initiated in Japan. The present study focused on the antitumor activity of paclitaxel against human breast carcinoma xenografts serially passaged in nude mice.

MATERIALS AND METHODS

Human Tumor Xenografts

Five human breast carcinoma xenografts, MCF-7, R-27, Br-10, T-61, and MX-1, were used. MCF-7 was

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established as a cultured cell line in 1970 by Soule et al. [13] and was successfully transplanted into nude mice treated with estrogen and progesterone in 1983 by the authors [14]. R-27 was also transplanted from a tamoxifen-resistant variant of MCF-7 [15] by the same procedure as that for MCF-7. Br-10 was established from floating cancer cells in the pleural effusion of a 43-year-old woman with an invasive ductal carcinoma in 1974 by Hirohashi et al. [16] and kindly supplied to our institute. T-61 was derived from cancerous tissue of a 54-year-old female patient with breast cancer and was kindly supplied by Dr. N. Brünner (Copenhagen University) [17]. MX-1 was established by Giovanella et al. [18] from cancerous tissue of a 29-year-old female patient and kindly supplied to our institute by Dr. K. Inoue (Cancer Chemotherapy Center, Tokyo), in 1979. These strains are histologically invasive ductal carcinomas, and all the xenografts were maintained in our laboratory as serially transplantable tumors in nude mice.

Nude Mice

Female nude mice with a BALB/cA genetic background were purchased from CLEA Japan (Tokyo). They were maintained under specific pathogen-free conditions using an Isorack in our experimental animal center and were fed sterile food and water ad libitum. Six- to eight-week-old mice weighing 20–22 g were used for the experiments.

Agents

Paclitaxel (BMS-181339: Taxol) and ethanol/Cremophor EL® (1:1 v/v) solution were obtained from Bristol-Myers Squibb K.K. (Tokyo).

For the nude mouse study, paclitaxel was dissolved at 30 mg/ml in ethanol/Cremophor EL® (polyethoxylated castor oil: glycerol polyethyleneglycol ricinoleate) solution, and stored at 4°C. This stock solution was further diluted to 2 mg/ml with 0.9% NaCl on the day of administration, and paclitaxel (20 mg/kg) in 0.2 ml of this ethanol/Cremophor EL® solution was given intraperitoneally daily for 5 days when the tumors started to grow exponentially.

Paclitaxel was also dissolved in dimethyl sulfoxide (DMSO: Nacalai Tesque, Kyoto, Japan) at a concentration of 10 mM (8.54 mg/ml) and stored at 4°C for the in vitro MTT assay. The maximum final concentration of DMSO was 50 μM, at which paclitaxel was not crystallized and no cytotoxicity of DMSO was observed.

Tumor Inoculation, Measurement of Tumor Size, and Evaluation of Agent Activity

Two fragments of tumor tissue, each measuring 3 × 3 × 3 mm, were inoculated into the subcutaneous tissue of the bilateral dorsum of ether-anesthetized nude mice, using a trocar needle. 17β-Estradiol dipropionate at 5 mg/kg and 17α-progesterone caproate at 250 mg/kg were injected intramuscularly into mice bearing MCF-7, R-27, and Br-10 on the day of tumor inoculation to ensure exponential tumor growth. The tumors were measured (length and width) with sliding calipers three times weekly by the same observer.

Tumor weight was calculated according to the method of Geran et al. [19] from linear measurements using the formula: tumor weight (mg) = length (mm) × (width (mm))^2/2. When the tumors reached 100–300 mg, the tumor-bearing mice were allocated randomly to test groups each consisting of between four and six mice. The relative mean tumor weight (RW) was calculated as RW = Wi/Wo, where Wi is the mean tumor weight at any given time and Wo is the mean tumor weight at the time of initial treatment. The antitumor effects of the agents were evaluated in terms of the lowest T/C value (%) during the experiment, where T is the relative mean tumor weight of the treated group and C the relative mean tumor weight of the control group at any given time. The antitumor activity was evaluated when the lowest T/C was equal to or less than 42%, which was calculated from (0.75)^3, corresponding to a 25% reduction of each diameter [20].

For MX-1, the regression equation was derived as ''the lowest T/C (as a percentage) = a − b × (dose in mg)'' and the minimum effective dose (MED) was calculated from the formula: MED = (a − 42)/b, where *a* and *b* are variables of the equation. The chemotherapeutic index was also calculated from the formula: 20 mg/kg (the maximum tolerated dose)/MED.

The mice were killed at the end of the experiments, and the tumors and spleens were then removed and weighed. The weights of the tumors and the spleens of the control and treated groups were compared by Student's t-test.

MTT Assay

The assay method of Mosmann [21] was applied with some modifications [22,23] reported previously. In brief, the xenografts were resected aseptically from nude mice, and single-cell suspensions were prepared enzymatically using 0.5 mg/ml pronase, 0.2 mg/ml collagenase type I, and 0.2 mg/ml DNase. After two centrifugations, tumor cells were suspended in RPMI-1640 medium supplemented with 20% FCS, diluted to 2 × 10^5–1 × 10^6 cells/ml, and then plated into 96-well microplates (Gibco, Gaithersburg, MD) in volumes of 50 μl. The paclitaxel/DMSO solution was dissolved in RPMI-1640 and added to the cell suspension in the wells at a volume of 50 μl.

TABLE I. Antitumor Activity of Paclitaxel (BMS-181339) Against Human Breast Carcinoma Xenografts

Tumor	Treatment[a]	No.[b]	Tumor weight[c]	T/C[d]	t[e]	T/C[f]
MCF-7	control	7	222.2 ± 58.1			
	5 mg/kg q.d. × 5	8	250.8 ± 106.7	112.8%	0.63	79.6%
	10 mg/kg q.d. × 5	5	258.8 ± 185.6	116.4%	0.50	81.1%
	20 mg/kg q.d. × 5	5	36.8 ± 15.4	16.6%	6.88*	11.6%
R-27	control	12	612.0 ± 233.0			
	10 mg/kg q.d. × 5	10	615.0 ± 147.0	100.5%	0.03	71.3%
	20 mg/kg q.d. × 5	12	535.0 ± 310.0	87.4%	0.68	66.1%
Br-10	control	9	1,207.6 ± 393.2			
	20 mg/kg q.d. × 5	8	1,381.5 ± 590.3	114.4%	0.69	95.4%
T-61	control	8	1,064.5 ± 401.1			
	20 mg/kg q.d. × 3	8	764.1 ± 781.5	71.8%	0.30	68.5%
T-61	control	7	1,350.6 ± 551.4			
	10 mg/kg q.d. × 5	8	1,204.3 ± 976.2	89.2%	0.35	98.9%
	20 mg/kg q.d. × 5	7	1,022.4 ± 492.9	75.7%	1.17	78.8%
MX-1	control	5	2,694.5 ± 1,663.8			
	20 mg/kg q.d. × 5	5	27.5 ± 61.4	0.1%	3.58**	1.0%
MX-1	control	5	2,072.0 ± 648.0			
	5 mg/kg q.d. × 5	5	1,425.0 ± 902.0	68.8%	1.30	62.7%
	10 mg/kg q.d. × 5	5	1,086.0 ± 367.0	52.4%	2.96***	48.4%
	10 mg/kg q.d. × 10	5	toxic			

[a]Paclitaxel (BMS-181339: Taxol) was dissolved in ethanol/Cremophor EL® (1:1 v/v) solution and given intraperitoneally daily at a volume of 0.2 ml/mouse.
[b]Number of tumors.
[c]Actual tumor weight in mg at the end of the experiments.
[d]T/C value of the actual tumor weight, where T is the actual mean tumor weight of the treated group and C the actual mean tumor weight of the control group at the end of experiments.
[e]*t*-value calculated by Student's *t* test.
*$P < 0.001$, **$P < 0.01$, ***$P < 0.02$.
[f]The lowest T/C value of the relative mean tumor weight during the experiments, where T is the relative mean tumor weight of the treated group and C the relative mean tumor weight of the control group at any given time. Underscore indicates positive antitumor activity.

Control wells were filled with 50 μl of cell suspension and 50 μl of RPMI-1640: 100 μl of RPMI-1640 with 10% FCS was plated as a blank. The plates were incubated for 96 h at 37°C in a humidified atmosphere containing 95% air and 5% CO_2. At the end of the incubation, 10 μl/well 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT, Sigma) dissolved in 5 mg/ml PBS and filtered through a 0.45 μm membrane filter (Millipore, Bedford, MA), and 0.1 M sodium succinate (Wako Pure Chemical Ind., Osaka, Japan) were added to form the formazan product. Following an additional 3-h incubation at 37°C, 150 μl per well of 99% DMSO were added to dissolve the formazan salt. After mixing with a pipette, absorbance of the fluid at a volume of 150 μl/well was read on a model EAR 340 Easy Reader (SLT-Labinstruments, Salzburg, Austria) at 540 and 630 nm, and the inhibition rate calculated using the formula: $(1 - A/B) \times 100\%$, where A and B represent the mean absorbance of the treated and control wells, respectively. The 50% inhibitory concentration (IC_{50}) was calculated from the formula: $IC_{50} = (\log 50 - a)/b$ when the linear regression equation between inhibition rate (IR) and drug concentration was $\log(IR) = a + b \times$ concentration.

Pharmacokinetics

Twenty mg/kg of paclitaxel was administered once intraperitoneally (ip) to R-27- or MX-1-bearing nude mice. The mice were killed at intervals, and the plasma and tumors were collected for pharmacokinetic assay by high-performance liquid chromatography [24,25]. The specimens were obtained at 1, 2, 4, 8, and 24 h after administration. At each point, the concentration in the tumor was determined using one to four mice each with one or two tumors. The concentration in plasma was the average for three to four mice, because the plasma concentration of paclitaxel was essentially the same in R-27- and MX-1-bearing nude mice.

RESULTS

The antitumor activity of paclitaxel on human breast carcinoma xenografts is shown in Table I. When 20 mg/kg paclitaxel was administered intraperitoneally (*ip*) daily for 5 days, the growth of MCF-7 and MX-1 was significantly inhibited, resulting in T/C ratios of 11.6% and 1.0%, respectively. The actual weight of these tumors was also significantly different from that of the control tumors at $P < 0.001$ and $P < 0.01$. Whereas the

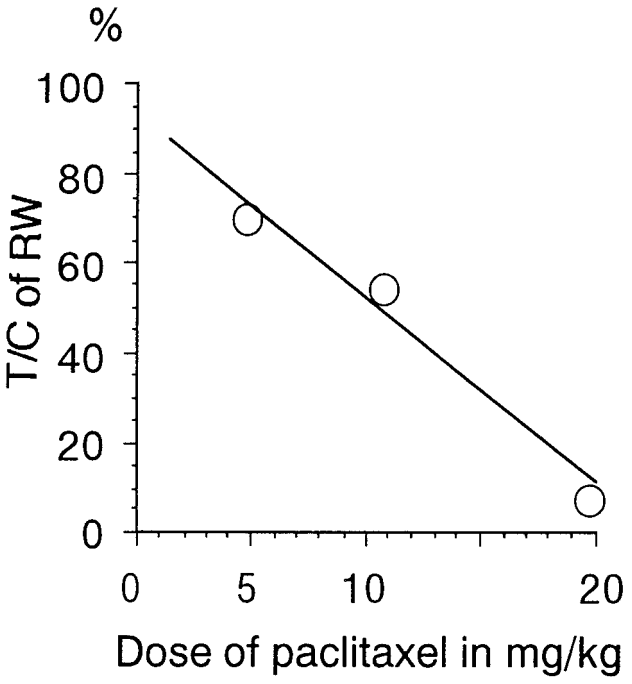

Fig. 1. Dose-dependent antitumor activity of paclitaxel on MX-1 a human breast carcinoma xenograft. The dose-dependency was linear with coefficient of correlation of 0.994 where T/C (%) = 86.4 − 4.203 × Dose (mg/kg). The calculated minimum effective dose (MED) was 10.6 mg/kg, giving a chemotherapeutic index of 20/10.6 (maximum tolerated dose/MED) = 1.887. T/C of RW: The relative mean tumor weight (RW) was calculated as RW = Wi/Wo, where Wi is the mean tumor weight at any given time and Wo is the mean tumor weight at the time of initial treatment. T/C was calculated as T/C = (RW of the treated group)/(RW of the control group) (%), and the lowest T/C value was shown in the figure. T/C = Treated/Control.

effect of paclitaxel on MX-1 at a dose of 10 mg/kg was marginal in terms of the relative mean tumor weight of 48.4%, the treated actual tumor weight was significantly different from the control tumor weight. Figure 1 shows the dose-dependent antitumor activity of paclitaxel on MX-1. The dose-dependency was linear and the calculated MED was 10.6 mg/kg, giving a chemotherapeutic index of 20/10.6 = 1.887.

Table II shows the side effects of paclitaxel in the tumor-bearing nude mice. Paclitaxel was toxic to MX-1, when this drug was administered at 10 mg/kg daily for 10 days, whereas the total administered dose was equivalent to a schedule of q.d. × 5 at a dose of 20 mg/kg, at which no toxic death or body weight loss of >20% was encountered. Thus daily intraperitoneal administration of paclitaxel at a dose of 20 mg/kg for 5 days seemed to be the maximum tolerated dose for BALB/c nude mice, allowing its maximal antitumor activity to be elucidated without severe toxicity.

MTT assay was performed using primarily cultured tumor cells from human breast carcinoma xenografts, of which the differing sensitivity to paclitaxel in vivo was successfully reproduced in vitro (Table III). A difference in the efficacy of the drugs was clearly revealed, the 50% inhibitory concentrations (IC_{50}) ranging from 13 μg/ml for MX-1 to 120 μg/ml for Br-10. The cutoff concentration of paclitaxel in the MTT assay was estimated to be 20 μg/ml, since the IC_{50} values of the in vivo sensitive MCF-7 and MX-1 were <15 μg/ml and those of the insensitive strains were >29 μg/ml.

The concentration of paclitaxel in plasma of nude mice bearing R-27 or MX-1, and R-27 and MX-1 tumors are shown in Table IV. Since there were no significant differences in the changes in the plasma concentration of paclitaxel with time in nude mice bearing R-27 or MX-1, the plasma data for the two groups were assessed together. Paclitaxel was rapidly distributed to R-27 and MX-1 tumors, and the sustained concentration ranged from 1 to 2 μg/g until 4 h after treatment. The shift of paclitaxel from plasma to tumor tissue was similar in R-27 and MX-1, and this intratumoral concentration was almost 1/10 of the cutoff concentration of paclitaxel in the MTT assay.

DISCUSSION

Paclitaxel is water-insoluble, and a preliminary preclinical study failed to elucidate its antitumor activity until ethanol/cremophor EL was adopted as the standard solvent [3]. Although in vitro study indicated that this cremophor can overcome the cross-resistance of multidrug-resistant tumor cells to paclitaxel, a previous study indicated that Cremophor-dissolved paclitaxel did not show significant increased antitumor activity against Adriamycin- and vincristine-resistant P388 leukemia in vivo [4]. Therefore in this study the antitumor activity of paclitaxel dissolved in ethanol/Cremophor EL was evaluated against human breast carcinoma xenografts using the *ip* administration route.

In the present study, paclitaxel was effective against MCF-7 and MX-1, but its antitumor activity against R-27, Br-10, and T-61 was limited. All these strains are human invasive ductal carcinomas, estrogen receptors (ER) were detected in these strains (except MX-1), and the tumor doubling times are reported as 15.9 ± 5.8 for MCF-7, 15.9 ± 4.4 for R-27, 10.8 ± 4.4 for Br-10, 9.5 ± 1.3 for T-61, and 6.4 ± 1.1 for MX-1 in days [26,27]. The growth of MX-1, a rapid-growing tumor without ER was completely inhibited by paclitaxel, whereas MCF-7, a slow-growing ER-positive strain was also sensitive to this agent. Since R-27 with a similar growth characteristic to MCF-7 was resistant to paclitaxel, the different sensitivity of these strains to paclitaxel was not completely explained from the growth characteristics or ER status.

And since no significant differences were observed in the pharmacokinetics of paclitaxel in sensitive (MX-1) and resistant (R-27) tumor lines, the efficacy of this agent

TABLE II. Side Effects of Paclitaxel (BMS-181339) Against Human Breast Carcinoma Xenografts in Nude Mice

Tumor	Treatment[a]	Death rate	Body wt. loss[b]	Spleen wt.[c]
MCF-7	control	0/5	No body wt. loss	
	5 mg/kg q.d. × 5	2/5	10.7%	79.3%
	10 mg/kg q.d. × 5	1/5	No body wt. loss	151.6%
	20 mg/kg q.d. × 5	1/5	1.9%	169.4%
R-27	control	0/7	1.3%	
	10 mg/kg q.d. × 5	0/7	No body wt. loss	101.8%
	20 mg/kg q.d. × 5	0/6	1.4%	105.3%
Br-10	control	0/5	No body wt. loss	
	20 mg/kg q.d. × 5	1/5	No body wt. loss	83.8%
T-61	control	1/5	No body wt. loss	
	20 mg/kg q.d. × 3	0/5	No body wt. loss	68.5%
T-61	control	0/4	No body wt. loss	
	10 mg/kg q.d. × 5	0/4	No body wt. loss	128.3%
	20 mg/kg q.d. × 5	0/4	No body wt. loss	115.1%
MX-1	control	0/5	No body wt. loss	
	20 mg/kg q.d. × 5	2/5	15.9%	ND
MX-1	control	3/5	No body wt. loss	
	5 mg/kg q.d. × 5	1/5	11.0%	ND
	10 mg/kg q.d. × 5	1/5	17.4%	ND
	10 mg/kg q.d. × 10	1/5	toxic	UD

[a]Paclitaxel (BMS-181339: Taxol) was dissolved in ethanol/Cremophor EL® (1:1 v/v) solution and given intraperitoneally daily at a volume of 0.2 ml/mouse.
[b]The maximum body weight loss during the experiments.
[c]The ratio of the treated/control mean spleen weight.
ND = not detected.
UD = undetectable.

TABLE III. In Vitro Sensitivity of Human Breast Cancer Strains to Paclitaxel Detected by MTT Assay

Conc.[a]	100	50	40	25	10	1	0.1	IC_{50}[b]
MCF-7	81[c]	78	75	ND	20	2	3	15
R-27	84	66	58	ND	18	10	10	29
Br-10	29	13	11	ND	4	2	0	120
T-61	59	ND	51	ND	22	8	1	38
MX-1	87	87	87	79	40	29	20	13

[a]Concentration (μg/ml) of paclitaxel in 3-(4.5-dimethy1–2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide assay (MTT assay).
[b]50% inhibitory concentration in μg/ml.
[c]Data shown as percentage inhibition rate.
ND = not done.

seemed to depend on the sensitivity of each type of tumor cell rather than the concentration of agent in tumor tissues. When the differences in sensitivity of the xenografts to paclitaxel in vivo were assessed by in vitro MTT assay with 96 h incubation, the cutoff concentration necessary to alter the chemosensitivity in vivo was estimated to be 20 μg/ml. Kiyozuka et al. [28] have reported paradoxical antitumor activity of paclitaxel in which a greater antitumor effect was obtained at a lower (0.074–0.94 μM) than at a higher (2.81 μM = 2.4 μg/ml) concentration in the MTT assay, due probably to a mitotic arrest effect at lower concentrations. The concentrations used in the present study, except for 0.1 and 1.0 μg/ml, were higher than their changing point at 2.4 μg/ml, and no paradoxical concentration dependency was observed. Furthermore, the cutoff concentration of paclitaxel determined from the MTT assay was 10-fold higher than the maximum concentration in plasma and tumors of mice injected *ip* with 20 mg/kg paclitaxel. This might be related to our previous data [22,23] showing that the cutoff concentrations were usually higher than the peak plasma concentrations for various drugs.

The antitumor spectrum of paclitaxel was compared with those of conventionally available antibreast cancer drugs, including mitomycin C, Adriamycin, cyclophosphamide, and hexamethylmelamine reported elsewhere [29,30] (Table V). The maximum tolerated doses and the schedules and routes of treatment with these agents are also shown in Table VI. The antitumor activity of paclitaxel on MX-1 was almost equivalent to that of hexamethylmelamine and cyclophosphamide, and paclitaxel was the most effective of these agents against MCF-7. Furthermore, paclitaxel was the only drug that showed different antitumor activities against MCF-7 and R-27,

TABLE IV. Concentration of Paclitaxel in Plasma and Tumors of R-27- or MX-1 Bearing Nude Mice†

Time[a]	Plasma (μg/ml)	n[b]	R-27 tumor[c]	n	MX-1 tumor[c]	n
1	1.380 ± 0.085	4	0.891 ± 0.218	4	0.268 ± 0.095*	8
2	1.543 ± 0.206	4	1.065 ± 0.298	4	0.494 ± 0.138**	4
4	0.632 ± 0.436	4	1.705 ± 0.571	4	1.681 ± 1.857	3
8	0.543 ± 0.016	4	0.912 ± 1.055	4	0.220 ± 0.004	2
24	<0.02	2	0.815 ± 0.092	2	0.182	1

†Twenty mg/kg of paclitaxel was administered once intraperitoneally (ip) to R-27- or MX-1-bearing nude mice, which were then killed at 1, 2, 4, 8, and 24 h after administration, and the plasma and tumors were collected for pharmacokinetic assay by high-performance liquid chromatography.
[a]Time after administration of paclitaxel at 20 mg/kg *ip.*
[b]Number of samples.
[c]Tumor concentration of paclitaxel in μg/g.
*$P < 0.001$, ** $P < 0.02$.

TABLE V. Antitumor Spectra of Various Agents Against Breast Carcinoma Xenografts in Nude Mice

Tumor	PCTL[a]	MMC[b]	ADM[c]	CPA[d]	HMM[e]
MCF-7	11.6[f]	23.4[g]	64.5	36.3	88.5
R-27	66.1	11.7	70.5	36.3	56.0
Br-10	95.4	21.0	90.0	30.9	40.6
T-61	78.8	44.1	52.0	21.4	0
MX-1	1.0	7.9	39.8	0.8	0
r[h]		0.45	0.66	0.48	0.12

[a]PCTL = paclitasel.
[b]Mitomycin.
[c]Adriamycin.
[d]Cyclophosphamide.
[e]Hexamethylmelamine.
[f]Data are shown as the lowest T/C value of the relative mean tumor weight during the experiments.
[g]Underscore indicates the positive antitumor activity, where the T/C value is <42%.
[h]Coefficient of correlation.

TABLE VI. Maximum Tolerated Doses, Schedules, and Administration Routes of the Drugs Tested Against Human Breast Carcinoma Xenografts

Drug[a]	Dose[b]	Schedule	Route
PCTL	20 mg/kg	q.d. × 5	intraperitoneal
MMC	6 mg/kg	q.d. × 1	intraperitoneal
ADM	8 mg/kg	q.d. × 1	intravenous
CPA	120 mg/kg	q.d. × 1	intraperitoneal
HMM	75 mg/kg	q.d. × 24	peroral

[a]See Table V for abbreviations.
[b]Maximum tolerated doses.

even though R-27 is a subline of MCF-7 [15] and its chemosensitivity is essentially identical to the parental line [29]. When the antitumor spectra of these agents were compared using the coefficients of correlation for the lowest T/C value, that of paclitaxel was substantially different from that of the conventionally available antibreast cancer drugs, in accord with our previous study, where the antitumor spectrum of KRN8602, an Adriamycin derivative, was found not to be essentially identical to that of Adriamycin [31]. This difference between the antitumor spectra of paclitaxel and conventionally available antibreast carcinoma agents suggests that paclitaxel would be useful against breast carcinomas resistant to the first line chemotherapy, including cyclophosphamide, Adriamycin, and mitomycin C.

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