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Anti-tumor Efficacy of Paclitaxel against Human Lung Cancer Xenografts

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We examined paclitaxel for anti-tumor activity against human lung cancer xenografts in nude mice and compared its efficacy with that of cisplatin, currently a key drug for lung cancer chemotherapy. Five non-small cell lung cancers (A549, NCI-H23, NCI-H226, NCI-H460 and NCI-H522) and 2 small cell lung cancers (DMS114 and DMS273) were chosen for this study, since these cell lines have been well characterized as regards *in vitro* and *in vivo* drug sensitivity. These cells were exposed to graded concentrations of paclitaxel (0.1 to 1000 n*M*) for 48 h. The 50% growth-inhibitory concentrations (GI_{50}) for the cell lines ranged from 4 to 24 n*M*, which are much lower than the achievable peak plasma concentration of paclitaxel. In the *in vivo* study, 4 cell lines (A549, NCI-H23, NCI-H460, DMS-273) were grown as subcutaneous tumor xenografts in nude mice. Paclitaxel was given intravenously as consecutive daily injections for 5 days at the doses of 24 and 12 mg/kg/day. Against every xenograft, paclitaxel produced a statistically significant tumor growth inhibition compared to the saline control. Paclitaxel at 24 mg/kg/day was more effective than cisplatin at 3 mg/kg/day with the same dosing schedule as above, although the toxicity of paclitaxel was similar to or rather lower than that of cisplatin, in terms of body weight loss. In addition, paclitaxel showed potent activity against 2 other lung cancer xenografts (NCI-H226 and DMS114). Therefore, paclitaxel showed more effective, wider-spectrum anti-tumor activity than cisplatin in this panel of 6 lung cancer xenografts. These findings support the potential utility of paclitaxel in the treatment of human lung cancer.

Key words: Paclitaxel — Human lung cancer — *in vitro* — *in vivo* — Anti-tumor efficacy

Paclitaxel (BMS-181339: Taxol) is a new anti-microtubular agent extracted from the bark of the Pacific yew (*Taxus brevifolia*).[1] It acts by promoting tubulin polymerization and stabilizing microtubules against depolymerization.[2,3] Paclitaxel has recently been approved for the treatment of refractory ovarian cancer and breast cancer in the United States, and furthermore, is expected to be applicable to other types of cancer.

In the chemotherapy of lung cancer, cisplatin is a key drug.[4] Recently, phase II studies of paclitaxel in non-small-cell lung cancer (NSCLC) have indicated high response rates.[5,6] Therefore, a paclitaxel-based regimen has been proposed to be a promising strategy for chemotherapy of lung cancer,[7] especially NSCLC that is highly resistant to available anti-cancer agents. To assess the potential of paclitaxel in lung cancer, preclinical studies to examine the anti-tumor activity of paclitaxel against lung cancer cell lines are required. There are some reports on the anti-tumor activity of paclitaxel against human lung cancer xenografts such as LX-1, H2981 and L2987,[8] and metastatic MV522.[9] However, these studies failed to assess the efficacy of paclitaxel in comparison with that of cisplatin.

The aim of this study was to examine the anti-tumor activity of paclitaxel against human lung cancer xenografts in nude mice in comparison with that of cisplatin, a reference anti lung cancer drug. We chose 5 NSCLC (A549, NCI-H23, NCI-H226, NCI-H460 and NCI-H522) and 2 small cell lung cancer (SCLC) lines (DMS114 and DMS273) for this study, since these cell lines have been well characterized as regards *in vitro* and *in vivo* drug sensitivity in the screening programs of the US National Cancer Institute[10] and the Japanese Foundation for Cancer Research.[11] Since paclitaxel inhibited the *in vitro* growth of the 7 human lung cancer cell lines at concentrations much lower than those achievable in plasma, we then examined its effect against 6 xenografts corresponding to the lung cancer cell lines in comparison with that of cisplatin.

MATERIALS AND METHODS

***In vitro* study** Five cell lines of NSCLC (A549, NCI-H23, NCI-H226, NCI-H460 and NCI-H522) and 2 cell lines of SCLC (DMS114 and DMS273) were used in this study. The histological types of NSCLCs were as follows; A549, NCI-H23 and NCI-H522 were adenocarcinomas, NCI-H226 was squamous cell carcinoma and NCI-H460 was large cell carcinoma. These NSCLCs and the SCLCs have been well characterized as regards *in vitro* and *in vivo* drug sensitivity in the screening programs of the US National Cancer Institute[10] and the Japanese Founda-

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tion for Cancer Research[11]; details of their origin and characterization were described elsewhere.[12]

The cells were plated at appropriate density in 96-well plates in RPMI-1640 medium with 10% fetal bovine serum, and allowed to attach overnight. Paclitaxel was solubilized in DMSO, and further diluted with RPMI-1640 medium. The cells in the wells were treated with the drug at concentrations of 0.1–1000 n*M*. After 48 h, cell growth was determined by means of the sulforhodamine B assay described by Skehan *et al.*[13] Data treatment followed the method described by Monks *et al.*[10] Briefly, optical densities of the control well (C) and the test well (T) was measured at 525 nm. Moreover, optical density was also measured at time 0 (the time when drugs were added) (T_0). Using these measurements, cell growth inhibition (percent growth) at each concentration of drug was calculated according to the following formulae: Percent growth $=100\times[(T-T_0)/(C-T_0)]$, when $T>T_0$. Percent growth $=100\times[(T-T_0)/T]$, when $T<T_0$. By computer processing of percent growth values, the 50% growth-inhibitory concentration (GI_{50}) was determined. The GI_{50} was calculated from $100\times[(T-T_0)/(C-T_0)]=50$.

Animals Female nude mice with a BALB/c genetic background were purchased from Charles River Japan, Inc. They were maintained under specific-pathogen-free conditions, and provided with sterile food and water *ad libitum*. Seven-week-old mice weighing 16–22 g were used for the present study.

Drugs and administration Drug administration was done intravenously. We selected an administration schedule of consecutive daily injections for 5 days (abbreviated as q1d×5) for paclitaxel because similar schedules were most effective in published experiments.[8, 14] We first investigated the maximum tolerable doses (MTD) of paclitaxel and cisplatin in the schedule of q1d×5, which proved to be 28 and 3 mg/kg/day, respectively. For the evaluation of anti-tumor effect, paclitaxel was given to tumor-bearing nude mice at a dose of 24 mg/kg/day, possibly the most effective dose according to a previous report[14] and at a suboptimal dose of 12 mg/kg/day. The reference drug cisplatin was given at the MTD (3 mg/kg/day), the most effective dose in our preliminary experiments. Paclitaxel was dissolved at 24 mg/ml in ethanol/Cremophor EL (1 : 1) solution, and stored at 4°C. This stock solution was diluted to 2.4 and 1.2 mg/ml with saline just before injection. Cisplatin was dissolved to 0.3 mg/ml in distilled water on the day of injection. Saline and paclitaxel diluent (ethanol/Cremophor EL/saline = 1 : 1 : 18) were also given to the control group and the vehicle control group, respectively.

Treatment and evaluation Four NSCLCs (A549, NCI-H23, NCI-H460 and NCI-H226) and 2 SCLCs (DMS114 and DMS273) were transplantable into nude mice, and were grown as subcutaneous tumor xenografts in nude mice. Nude mice were inoculated subcutaneously with a 3×3×3 mm tumor fragment. When the tumor reached 100–300 mm^3 in volume, animals were divided randomly into test groups consisting of 6 mice per group (day 0). Drugs were administered from day 0. The mice were weighed twice a week up to day 24–31 to monitor the toxic effects. The length (L) and width (W) of the tumor mass were measured twice a week up to day 24–31, and the tumor volume (TV) was calculated according to the following formula: $TV=(L\times W^2)/2$. Each tumor volume at day n was expressed as relative tumor volume (RTV) according to the following formula: $RTV=TV_n/TV_0$, where TV_n is the tumor volume at day n, and TV_0 is the tumor volume at day 0. Tumor regression (T/C%) at day 14 was determined by calculating RTV according to the following formula: T/C% = 100×(mean RTV of treated group)/(mean RTV of control group). Statistical evaluations of RTV were performed using the Mann Whitney U-test.

RESULTS

***In vitro* growth inhibition of human lung cancer cells by paclitaxel** Seven human lung cancer cell lines including 5 NSCLCs (A549, NCI-H23, NCI-H226, NCI-H460 and NCI-H522) and 2 SCLCs (DMS114 and DMS273) were exposed to graded concentrations of paclitaxel (0.1 to 1000 n*M*) for 48 h. Their cell growth was inhibited in a concentration-dependent manner. The 50% growth-inhibitory concentrations (GI_{50}) for the cell lines ranged from 4 to 24 n*M*, and the mean GI_{50} was 9.9 n*M* (8.5 ng/ml) (Table I). This concentration is much lower than the GI_{50}s of cisplatin for these cell lines (mean of the GI_{50}s, 5.7 μM, experimental data not shown).

Anti-tumor efficacy of paclitaxel against human lung cancer xenografts The human lung cancer cell lines used

Table I. *In vitro* Growth-inhibitory Activity of Paclitaxel against Human Lung Cancer Cells

Cell line	GI_{50} (n*M*)
NCI-H23	9.8
NCI-H226	24.0
NCI-H522	4.0
NCI-H460	4.8
A549	19.0
DMS273	3.6
DMS114	4.1
Mean±SD	9.9±8.3

Lung cancer cells were exposed to paclitaxel at concentrations of 0.1–1000 n*M* for 48 h, and the cell growth inhibition was determined by means of the sulforhodamine B assay.

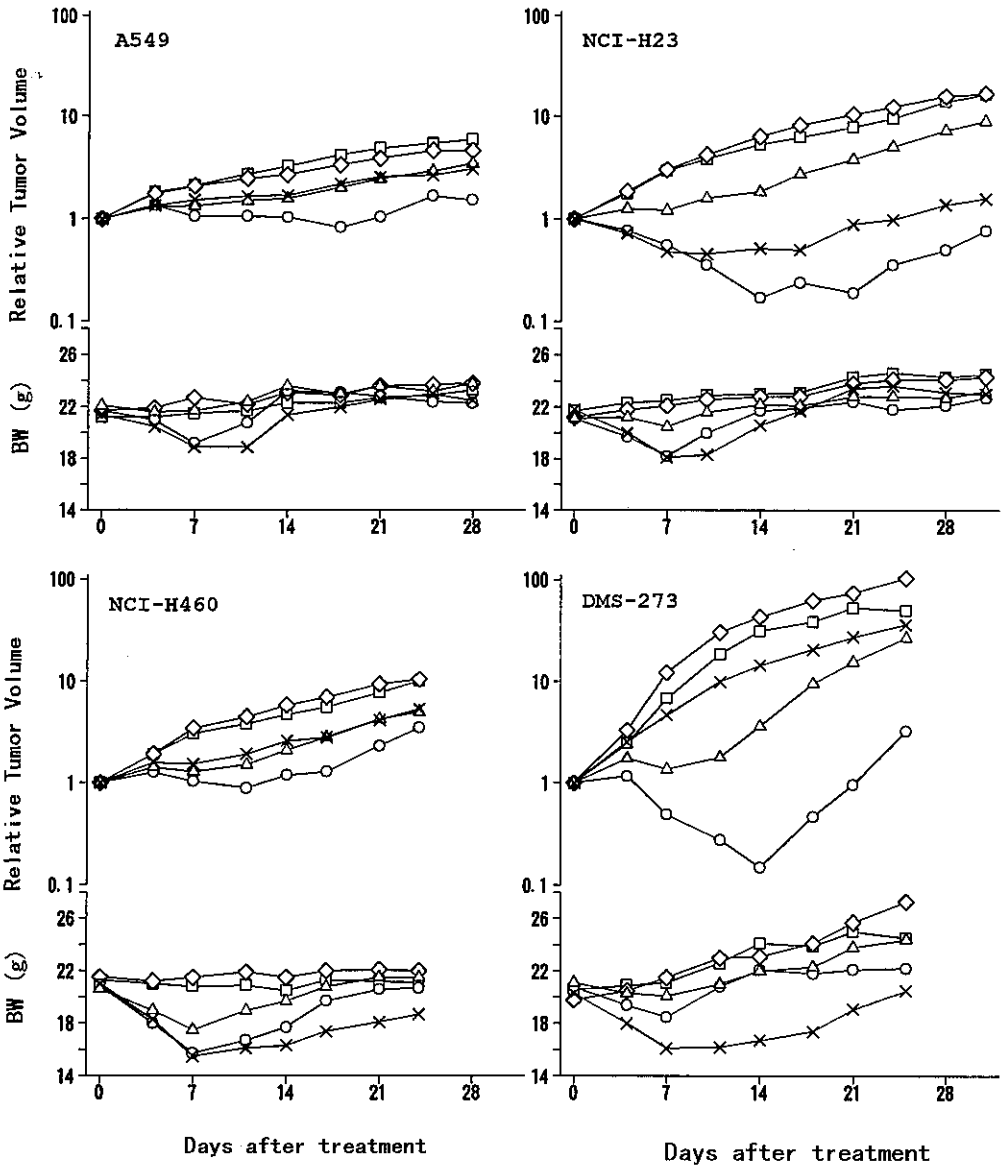

Fig. 1. Tumor growth and body weight (BW) change in nude mice bearing human lung cancer xenografts and administered paclitaxel. Nude mice were inoculated subcutaneously with a 3×3×3 mm tumor fragment. When the tumor reached 100–300 mm^3 in volume, animals were divided randomly into test groups consisting of 6 mice per group (day 0). Drugs were administered from day 0 with the schedule of q1d×5, saline (□), paclitaxel diluent (◇), paclitaxel at 24 (○) and 12 mg/kg/day (△), and cisplatin at 3 mg/kg/day (×). There were no spontaneous disappearance of the tumor in any of the control mice. Each curve represents the average of six mice.

for the *in vitro* study are transplantable into nude mice, except for NCI-H522. Therefore, we examined the activity of paclitaxel against the 6 lung cancer xenografts, i.e., 4 NSCLCs (A549, NCI-H23, NCI-H226 and NCI-H460) and 2 SCLCs (DMS114 and DMS273). The xenografts were grown as subcutaneous tumors in nude mice, and the administration of drugs was started with the schedule of q1d×5 when the tumor reached 100–300 mm^3 in volume. The maximum tolerable doses of paclitaxel and cisplatin in this administration schedule are 28 and 3 mg/kg, respectively.

First, we compared paclitaxel at doses of 24 and 12 mg/kg with cisplatin at the dose of 3 mg/kg for antitumor activity against four of the lung cancer xenografts,

Table II. Anti-tumor Efficacy of Paclitaxel against Human Lung Cancer Xenografts on Day 14

Tumor	Drug[a)]	Dose (mg/kg/day)	Mean±SD of RTV[b)]	T/C%[c)]	Max. Dec. (%) in BW[f)]
A549	Saline	0	3.3±1.0	100	—
	Diluent[g)]	0	2.7±0.4	83	ND[h)]
	Paclitaxel	24	1.0±0.5	32[d, e)]	12
	Paclitaxel	12	1.6±0.2	49[d)]	2
	Cisplatin	3	1.7±0.5	52	12
NCI-H23	Saline	0	5.3±1.0	100	—
	Diluent	0	6.4±2.2	121	ND
	Paclitaxel	24	0.2±0.1	3[d, e)]	14
	Paclitaxel	12	1.8±0.5	35[d)]	3
	Cisplatin	3	0.5±0.3	10[d)]	17
NCI-H460	Saline	0	4.7±0.9	100	—
	Diluent	0	5.8±1.1	123	1
	Paclitaxel	24	1.2±0.5	25[d, e)]	25
	Paclitaxel	12	2.1±0.3	45[d)]	15
	Cisplatin	3	2.6±0.9	55[d)]	26
DMS273	Saline	0	31.3±18.8	100	—
	Diluent	0	43.0±26.3	137	ND
	Paclitaxel	24	0.2±0.1	0[d, e)]	11
	Paclitaxel	12	3.6±2.1	12[d, e)]	5
	Cisplatin	3	14.4±6.0	46	21
NCI-H226	None	0	3.8±0.8	100	—
	Paclitaxel	28	0.2±0.2	6[d)]	20
DMS114	None	0	7.3±3.9	100	—
	Paclitaxel	28	2.0±0.8	27[d)]	11

a) Nude mice were inoculated subcutaneously with a 3×3×3 mm tumor fragment. When the tumor reached 100–300 mm^3 in volume, animals were divided randomly into test groups consisting of 6 mice per group (day 0). Drugs were administered from day 0 with the schedule of q1d×5.
b) Relative tumor volume: tumor volume at day 14/tumor volume at day 0.
c) Tumor regression: 100×mean RTV of treated group/mean RTV of control group.
d) Significantly different from control group (Saline or None), $P<0.01$ by Mann Whitney U-test.
e) Significantly different from cisplatin group, $P<0.01$ by Mann Whitney U-test.
f) Maximum decrease (%) in body weight (BW): 100×(the starting BW−the BW showing maximum decrease)/the starting BW.
g) Paclitaxel diluent: ethanol/Cremophor EL/saline (1 : 1 : 18).
h) No decrease in body weight.

A549, NCI-H23, NCI-H460 and DMS273. The tumor growth and the body weight changes are illustrated in Fig. 1, and the efficacy of the drugs determined at day 14 is summarized in Table II. Paclitaxel significantly inhibited the growth of all 4 tumors at both doses, and its effect was dose-dependent. At day 14, T/C% values in mice treated with 24 mg/kg of paclitaxel were 32% for A549, 3% for NCI-H23, 25% for NCI-H460 and 0% for DMS273, each of which was significantly lower than the T/C% in mice treated with 3 mg/kg of cisplatin: 52% for A549; 10% for NCI-H23; 55% for NCI-H460 and 46% for DMS273 (Table II). In particular, 24 mg/kg of paclitaxel induced tumor regression in NCI-H23 and DMS273 with a duration of at least 10 days (Fig. 1). The anti-tumor activity of paclitaxel at a dose of 28 mg/kg against xenografts of NCI-H226 and DMS114 was also investigated and the results are shown in Table II. These two xenografts also showed remarkable responses to paclitaxel. In contrast, they were insensitive to cisplatin when administered at a dose of 10 mg/kg in one shot (the T/C values were 71% for NCI-H226 and 69% for DMS114). We previously confirmed that the 10 mg/kg of cisplatin in one shot had almost the same efficacy against A549, NCI-H23, NCI-H226 and NCI-H460 as 3 mg/kg of cisplatin with the schedule of q1d×5 (data not shown). Therefore, paclitaxel showed potent anti-tumor effects against all 6 lung cancer xenografts tested.

Administration of paclitaxel at doses of 12–28 mg/kg with the schedule of q1d×5 caused no toxic death of tumor-bearing mice during the monitoring period up to day 24–31. Maximum decrease in body weight induced by paclitaxel treatment appeared around day 7 and was

in the range of 11 to 25%, which was comparable to or rather lower than that induced by cisplatin treatment (Fig. 1 and Table II). The recovery rate from the decreased body weight was clearly faster in paclitaxel-treated mice than in cisplatin-treated mice (Fig. 1). These results demonstrate that paclitaxel is superior to cisplatin in therapeutic efficacy against lung cancer xenografts in nude mice.

DISCUSSION

We conducted the present study to examine the anti-tumor activity of paclitaxel against human lung cancer xenografts in nude mice and to compare its therapeutic efficacy with that of cisplatin. We chose 7 well-characterized human cancer cell lines for this study. First, we investigated the concentrations of paclitaxel that inhibit the *in vitro* proliferation of these lung cancer cells. The GI_{50}s of paclitaxel for the cell lines fell in the narrow range of 4 to 24 n*M*, indicating that these cell lines all have rather similar sensitivities to paclitaxel. The p53 status may be an important factor for chemosensitivity, as mentioned by Perego *et al.*[15] and Wahl *et al.*[16] We detected mutation of p53 in exon 5, 6, 7 or 8 in three of the lung cancer cell lines (NCI-H23, DMS273 and DMS114), but not in others (NCI-H226, NCI-H522, NCI-H460 and A549) (unpublished results). Therefore, the p53 status in the lung cancer cell lines examined here did not show a clear correlation with paclitaxel sensitivity, as reported by Weinstein *et al.*[17]

The mean GI_{50} of paclitaxel for these cell lines (9.9 n*M*, 8.5 ng/ml) was very much lower than that of cisplatin (5700 n*M*, 1700 ng/ml). Similar differences between the two drugs in ovarian cancer cell lines were described by Engblom *et al.*[18] The mean GI_{50} of paclitaxel is considerably (at least 100-fold) lower than the peak plasma concentration (5–9 μM) achieved by 3-h infusion of paclitaxel in a phase I study[19] or the maximum concentration in plasma (1.8 μM) and tumor (2.0 μM) of mice after the injection of 20 mg/kg paclitaxel.[20] Therefore, we considered that plasma concentrations of paclitaxel exceeding the above GI_{50}s would be easily achievable in nude mice, and were encouraged to assess the anti-tumor activity of paclitaxel against human lung cancer xenografts in nude mice.

Anti-tumor activity of paclitaxel *in vivo* had been demonstrated in only a few tumor models in initial studies[8, 21, 22] because of the water-insoluble character of the drug. Subsequently, the use of ethanol/Cremophor EL as a vehicle[8] facilitated preclinical studies evaluating paclitaxel in various *in vivo* tumor models.[8,14] According to the report of Rose,[8] human cancer xenografts, including LX-1, H2981 and L2987 lung cancers, in nude mice responded to paclitaxel intravenously administered at 12–40 mg/kg for 2–5 days. However, no comparative study was done with reference drugs and therefore, the superiority of paclitaxel was not objectively determined. In a metastatic lung cancer xenograft model of lung cancer cell line MV522,[9] both mitomycin C and paclitaxel at toxic dose levels displayed marginal efficacy.

In this *in vivo* study, paclitaxel was dissolved in ethanol/Cremophor EL solution and intravenously administered to tumor-bearing nude mice with the schedule of q1dx5. First, paclitaxel was evaluated for anti-tumor activity against 4 human lung cancer xenografts (A549, NCI-H23, NCI-H460 and DMS273) in comparison with cisplatin, currently a key drug in lung cancer chemotherapy.[4] Paclitaxel at a dose of 24 mg/kg achieved significantly lower T/C% values than cisplatin (3 mg/kg) for all tumors, although its toxicity was similar to or rather lower than that of cisplatin, in terms of body weight loss. Furthermore, paclitaxel showed potent activity against two other lung cancer xenografts, NCI-H226 and DMS114, that were insensitive to cisplatin. Thus, paclitaxel displayed superior therapeutic efficacy to cisplatin against all the lung cancer xenografts tested. The large difference between the GI_{50} and the MTD of paclitaxel may contribute to this *in vivo* therapeutic efficacy. However, it remains unclear why the *in vivo* effective doses of paclitaxel were rather close to those of cisplatin while the *in vitro* GI_{50}s of paclitaxel were very much lower than those of cisplatin. Differences in tissue distribution and in pharmakokinetics might be related to these results.

Our findings support the potential value of paclitaxel for the clinical treatment of human lung cancer, and suggest that a paclitaxel-based regimen could be a promising strategy for chemotherapy of lung cancer. However, further preclinical and clinical investigations are needed to optimize its therapeutic efficacy. Moreover, evaluating the therapeutic efficacy of paclitaxel in combination with cisplatin may be important, as the two drugs obviously differ in their modes of action.

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