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Evaluation of antitumor activity of navelbine (vinorelbine ditartrate) against human breast carcinoma xenografts based on its pharmacokinetics in nude mice

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The *in vitro* antitumor activity of navelbine (NVB, KW-2307), a newly synthesized vinca alkaloid, was compared with that of adriamycin (ADM) against human breast carcinomas inoculated into nude mice at the maximum tolerated dose (MTD) and clinically equivalent dose (CED). The plasma levels of NVB after intravenous injection into nude mice at doses of 1.2 and 4.8 mg/kg diminished rapidly during the early phase (0–1 h), followed by a very long shallow one. NVB was still detected 96 h after administration at a dose of 4.8 mg/kg. The pharmacokinetic parameters of NVB in plasma indicated that NVB extensively distributes to tissues. The CED of NVB was provisionally decided to be 4.8 mg/kg based on the comparison of AUC values at 24–∞ h between human patients and nude mice. When compared by a single injection of MTD (NVB, 16 mg/kg; ADM 12 mg/kg), NVB was effective against all four tumor lines, MC-2, MC-8, MMKY and H-31, while ADM was effective only against H-31. On the other hand, the body weight loss by NVB was mild as compared with that by ADM, indicating that the antitumor activity of NVB is superior to that of ADM at their MTDs. A single injection of NVB at its CED (4.8 mg/kg) produced a poor antitumor effect and no or little toxicity in terms of body weight loss, as compared with those at MTD. However, when NVB was administered intermittently at CED, it exhibited significant antitumor activity against three tumor lines. The body weight loss was still mild even on this intermittent schedule. These results indicate that NVB can offer antitumor activity against human breast carcinoma xenografts at its CED.

Key words: Navelbine, pharmacokinetics, xenograft.

Introduction

The human tumor–nude mouse system has been proved to be a useful model to evaluate the activity of various antitumor agents.[1–5] However, the correlation of their efficacy in this system with that in clinical studies is not necessarily satisfactory and the predictability of clinical efficacy still remains to be established. One of the major reasons for such unsatisfactory correlation is a difference of the maximum tolerated dose (MTD) and pharmacokinetics of antitumor agents between nude mice and human patients. Based on these considerations, the concept of the 'clinically equivalent dose (CED)' was presented to improve the clinical predictability of the human tumor–nude mouse system.[6–9] The CED is defined as a dose of drugs for therapeutic experiments, which can reproduce their clinically achievable plasma levels in mice. The therapeutic efficacy at CED was proved to give better correlation with the clinical response rates in stomach, breast and lung cancer xenograft models.[7–9]

Navelbine (NVB, vinorelbine ditartrate, KW-2307) is a new vinca alkaloid analog synthesized by Potier *et al.*[10,11] NVB showed antitumor activity superior to vincristine, vinblastine or vindesine in human tumor models inoculated into nude mice, including non-small cell lung carcinomas and breast carcinomas.[12,13] Based on these results, clinical studies of NVB have been conducted in many institutes and, so far, its efficacy has been established against non-small cell lung cancer[14–16] and advanced breast cancer.[17] The combination chemotherapy of NVB plus cisplatin has been proved to be rational experimentally against non-small cell lung carcinomas in both *in vitro* and *in vivo* studies.[18,19] Concerning breast cancer, the combination chemotherapy of NVB plus adriamycin (ADM) offered better results and a response rate of 74% was reported.[20,21]

The present study was carried out in order to compare the antitumor activity of NVB with that

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of ADM against human breast carcinomas inoculated into nude mice in terms of CED.

Materials and methods

Chemicals

NVB and ADM were prepared by Kyowa Hakko Kogyo (Tokyo, Japan). These compounds were dissolved in sterile 0.9% NaCl solution. The dose of NVB was expressed based on the weight of the free base.

Animals

Female BALB/c-*nu/nu* mice (hereinafter designated as nude mice, 6–7 weeks old) weighing 20–25 g were obtained from CLEA Japan (Tokyo, Japan).

Antitumor activity

Human breast carcinomas, MC-2, MC-8, MMKY and H-31, were maintained at the Central Institute for Experimental Animals (Kawasaki, Japan).[8] The tumor fragments (8 mm^3) were inoculated subcutaneously into the flank of nude mice. When tumor volume was between 100 and 300 mm^3, the mice were randomized to six experimental groups consisting of six animals per group and drugs were injected intravenously according to the indicated schedule. The lengths and widths of tumors were measured twice a week, and tumor volume was calculated by using the following formula according to the method of the National Cancer Institute:[22]

$$\text{tumor volume } (mm^3) = \frac{\text{length (mm)} \times [\text{width (mm)}]^2}{2}.$$

The tumor growth rate was expressed as the mean V/V_0 value, where V is the tumor volume on the day of evaluation and V_0 is that on the starting day of treatment. The T/C value was calculated by the mean V/V_0 value of the treated group versus that of the control group.

The experimental results were analyzed by the Mann–Whitney U-test for statistical significance.

Determination of NVB concentration in plasma

Female nude mice (6 weeks old) weighing 20–25 g were housed under conditions of controlled temperature and lighting (12 h), and had free access to food and water at all times. NVB (1.2 or 4.8 mg/kg) was injected intravenously into nude mice and blood was collected at the indicated time after dosing. The concentrations of NVB in plasma were determined by radioimmunoassay (RIA) with a modification of a previous method.[23] Briefly, plasma was diluted, if necessary, in phosphate-buffered (10 mM, pH 7.4) saline containing 0.1% bovine serum albumin (Fraction V; Sigma, St Louis, MO) and incubated with antiserum and [^{125}I]NVB glycyl-L-tyrosine conjugate at 4°C for 4 h. At the end of the incubation, dextran-coated charcoal suspension [4% dextran T-70 (Pharmacia, Uppsala, Sweden), 2% charcol (Wako Pure Chemical, Osaka, Japan)] was added. Supernatant was separated by centrifugation (2700 r.p.m., 15 min) and counted for 1 min on a gamma counter (CRYSTAL; Packard, Meriden, CT). NVB concentrations were determined by using a standard curve (detection limit, 0.5 ng/ml).

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using model-independent methods.[24] The terminal rate constant was determined by log–linear regression analysis of the terminal phase of the plasma concentration–time curves. The terminal plasma half-lives were calculated as half-life equals 0.693/terminal rate constant. The area under the concentration–time curve (AUC) and the area under the first-moment curve (AUMC) were calculated by the trapezoidal rule with extrapolation to infinity. The mean residence time (MRT) was calculated by dividing the AUMC by the AUC. Total plasma clearance (Cl_{tot}) was calculated by dividing the dose by the AUC extrapolated from zero to infinity. The distribution volume at steady state (V_{dss}) was determined as $V_{dss} = Cl_{tot} \times MRT$.

Results

Plasma concentration of NVB in nude mice

The plasma levels of NVB after intravenous injection into nude mice are shown in Figure 1. The doses of NVB were chosen by considering that CED values of other vinca alkaloids were much smaller than their MTD values.[6] The concentration of NVB was measured by RIA, since it was used for the determination of NVB concentration in plasma of human patients in a phase I study.[25] The plasma

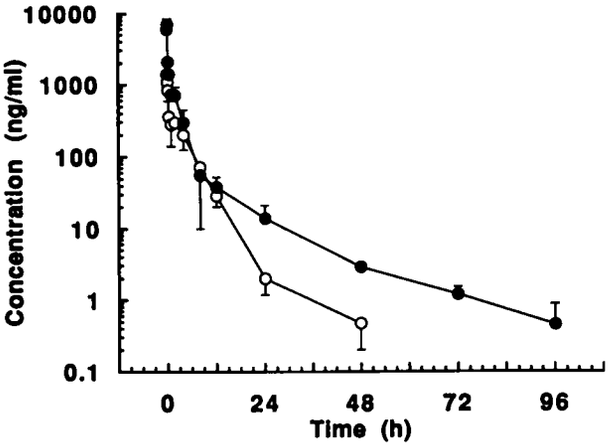

Figure 1. Plasma concentration of NVB in normal nude mice: 1.2 (○) or 4.8 (●) mg/kg of NVB was administered i.v. Plasma concentrations were determined by RIA. Points, mean values; bars, SD (n = 3).

concentrations observed at the first sampling time (3 min after administration) were 720–1900 ng/ml at a dose of 1.2 mg/kg and 4000–8600 ng/ml at a dose of 4.8 mg/kg, respectively. The NVB concentration diminished rapidly from the plasma during the early phase (0–1 h), followed by a very long shallow one. NVB was still detected 96 h after administration at a dose of 4.8 mg/kg, and mean concentrations after 24, 48, 72 and 96 h were 14, 2.9, 1.2 and 0.46 ng/ml, respectively. The pharmacokinetic parameters of NVB in plasma were calculated by model-independent analysis and summarized in Table 1. The dose dependency of AUC varied slightly at individual intervals as 0–∞, 12–∞ and 24–∞ h for the calculation of AUC. The large V_{dss} values indicate that NVB extensively distributed to tissues.

NVB concentrations in the plasma of cancer patients at a dose of 25 mg/m^2 in a phase I study were 5.9 and 4.9 ng/ml after 24 and 48 h, respectively.[25] AUC values were 1140 and 260 ng h/ml at the intervals of 0–∞ and 24–∞ h, respectively. The formula for the calculation of the CED has not been fully established in the case of mitotic inhibitors, of which antitumor activity is time-dependent and augmented significantly by an elongation of exposure time.

Thus, we supposed that the plasma concentration of NVB after 24 h was more important to offer therapeutic responses because the cell growth-inhibitory activity of NVB was significantly augmented at the exposure time between 8 and 24 h.[18] Therefore, we provisionally regarded a dose of 4.8 mg/kg of NVB as its CED in nude mice based on the comparison of its AUC (24–∞ h) values between human patients and nude mice.

Antitumor activity against human breast carcinomas

The antitumor activity of NVB was compared with that of ADM at both the MTD and CED. The characteristics of four human breast carcinomas used in this experiment are shown in Table 2. No toxic death mice were observed in all treatment groups (Table 3). When compared at the MTD (NVB, 16 mg/kg; ADM 12 mg/kg), NVB was effective against all four tumor lines, while ADM was effective only against H-31. On the other hand, body weight loss of over 2 g/mouse by NVB was observed only in MMKY-bearing mice, while that by ADM in tumor-bearing mice was seen in three lines. These results indicate that the antitumor activity of NVB is superior to that of ADM at their MTDs. The growth curves of tumors treated with NVB or ADM are shown in Figures 2 and 3. NVB (16 mg/kg) significantly inhibited the growth of all tumor lines and tumor regression was observed in MC-8- and MMKY-bearing mice between 1 and 2 weeks after administration.

A single administration of NVB at its CED (4.8 mg/kg) produced a poor antitumor effect and no or little

Table 1. Pharmacokinetic parameters of NVB in plasma after intravenous administration into nude mice

Dose (mg/kg)	$T_{1/2}$ (h)			AUC (ng h/ml)			MRT (h)	Cl_{tot} (l/h/kg)	V_{dss} (l/kg)
	π	α	β	0—∞	12–∞	24–∞			
1.2[a]	0.171	2.78	11.7	2350	220	37	4.43	0.512	2.27
4.8[b]	0.115	1.86	14.9	5530	600	280	4.57	0.868	3.97

The values were calculated from mean plasma concentrations of five mice in a group.
[a] π phase, 0.05–1 h; α phase, 2–12 h; β phase, 24–48 h.
[b] π phase, 0.05–1 h; α phase, 2–12 h; β phase, 24–96 h.

Table 2. Profile of human breast carcinoma xenograft lines

Tumor lines	Histological type	Tumor mass doubling time (day)	Hormone receptor	
			ER[a]	PgR[b]
MC-2	medullary tubular adenocarcinoma	8.9	—	—
MC-8	medullary tubular adenocarcinoma	7.1	—	—
MMKY	medullary tubular adenocarcinoma	14.4	—	—
H-31	papillotubular adenocarcinoma	7.8	—	—

[a] Estrogen receptor.
[b] Progesterone receptor.

Table 3. Antitumor activity of NVB or ADM against human breast carcinomas inoculated into nude mice

Tumors	Drugs	Dose (mg/kg)	Schedule (on day)	*T/C* (%) on day 14	Maximum body weight change (g)
MC-2	NVB	16	0	32[a]	−0.6
		4.8	0	87	no reduction
		4.8	0,4,8	64	no reduction
	ADM	12	0	57	−2.4
		8.8	0	82	−0.3
MC-8	NVB	16	0	13[a]	−1.0
		4.8	0	81	no reduction
		4.8	0,4,8	35[a]	−0.3
	ADM	12	0	62	−2.1
		8.8	0	70	−1.7
MMKY	NVB	16	0	43[a]	−2.7
		4.8	0	86	−0.2
		4.8	0,4,8	75	−2.0
	ADM	12	0	78	−2.0
		8.8	0	85	−1.4
H-31	NVB	16	0	36[a]	−0.7
		4.8	0	78	no reduction
		4.8	0,4,8	81	no reduction
	ADM	12	0	43[a]	−1.8
		8.8	0	60	−0.8

NVB or ADM was administered i.v. into nude mice following each schedule. Mortality was observed for 14 days.
[a] *T/C* (%) ≤ 50 and $p < 0.01$ (one-sided) versus untreated group by Mann–Whitney *U*-test.

toxicity in terms of body weight loss, as compared with those at the MTD (Table 3). However, when NVB was administered intermittently at the CED, its antitumor activity was augmented and statistically significant against MC-2, MC-8 and MMKY (Figures 2 and 3). Body weight loss was still mild even in this intermittent schedule. The antitumor activity of ADM reduced at its CED (8.8 mg/kg) as compared with that at the MTD (12 mg/kg).

Discussion

We have previously demonstrated that NVB exhibited significant growth-inhibitory activity at its MTD (16 mg/kg) against human tumors inoculated into nude mice, including four non-small cell lung carcinoma lines and three breast carcinoma lines.[13] Such activity of NVB was also confirmed in this study against an additional four human breast carcinoma lines (Table 3). Tumor regression was observed in MC-8- and MMKY-bearing mice (Figures 2 and 3), and, furthermore, body weight loss of nude mice administered with NVB was mild (Table 3). These activities of NVB were superior to those of ADM, which is widely used for the clinical therapy of breast cancers. However, the results obtained at their MTDs in nude mice do not always correlate with clinical responses, because MTD values of antitumor agents are often much smaller in human

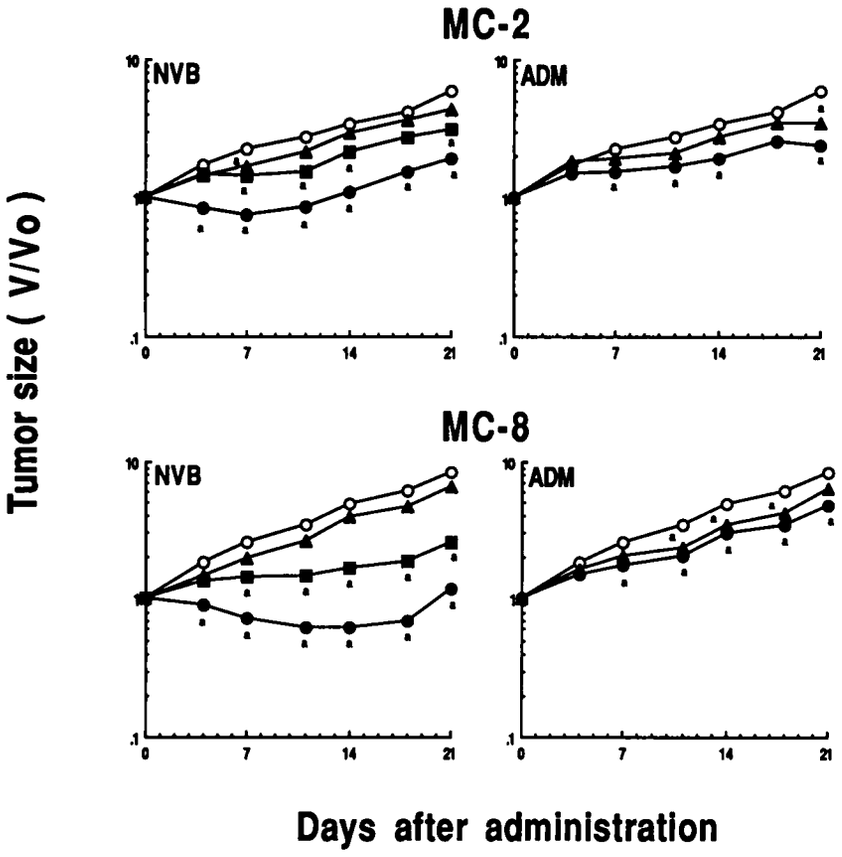

Figure 2. Growth pattern of human breast carcinomas inoculated into nude mice. Human breast carcinomas MC-2 and MC-8 were treated as described in Table 3: ○, untreated; ●, NVB 16 mg/kg or ADM 12 mg/kg on day 0; ▲, NVB 4.8 mg/kg or ADM 8.8 mg/kg on day 0; ■, NVB 4.8 mg/kg on days 0, 4 and 8.
[a]$p < 0.01$ (one-sided) versus untreated group by Mann–Whitney *U*-test.

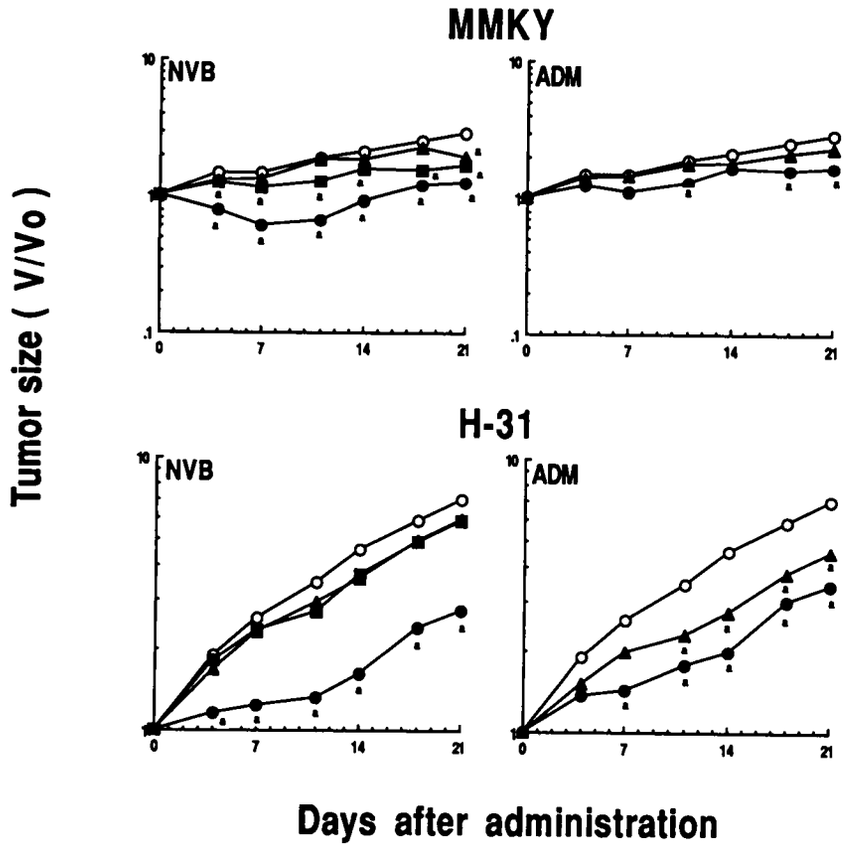

Figure 3. Growth pattern of human breast carcinomas inoculated into nude mice. Human breast carcinomas MMKY and H-31 were treated as described in Table 3: ○, untreated; ●, NVB 16 mg/kg or ADM 12 mg/kg on day 0; ▲, NVB 4.8 mg/kg or ADM 8.8 mg/kg on day 0; ■, NVB 4.8 mg/kg on days 0, 4 and 8. [a]$p < 0.01$ (one-sided) versus untreated group Mann–Whitney *U*-test.

patients than in mice. This is considered to be one of the major reasons why experimental therapeutic results do not fully predict clinical efficacy.[6]

Therefore, the concept of the CED was presented and CED values of various drugs were obtained.[6] The CED is defined as the dose of drugs for therapeutic experiments, which can reproduce their clinically achievable plasma levels in mice. Concerning vinca alkaloids, the MTDs were approximately 4-fold greater than the CEDs in nude mice. This was considered to be one of the possible reasons for their restricted clinical usefulness, although they exhibited remarkable antitumor activity against almost all kinds of tumors at MTD in human tumor–nude mouse models. However, the formula for calculation of the CED has not been fully established in the case of mitotic inhibitors, since the characteristics of their cell growth-inhibitory activity are greatly time-dependent and their plasma concentration at the late phase is considered to be more important than the peak plasma concentration for antitumor activity. Since the cell growth-inhibitory activity of NVB significantly increased at exposure times between 8 and 24 h,[18] we provisionally decided the CED of NVB by comparing the AUC values at 24–∞ h in both human patients and nude mice (Table 1). A single injection of NVB at the CED produced insignificant antitumor activity, as compared with that at the MTD (Table 3). In clinical treatments, NVB is administered weekly and its efficacy became detectable after several courses.[14–17] Therefore, we compared the antitumor activity of NVB at the CED between single and intermittent schedules, and found that its activity became detectable by intermittent treatment. These results suggested that NVB is expected to show antitumor activity against breast carcinomas clinically and it has been actually proved in clinical studies.[17,20,21] We previously reported that NVB was effective at inhibiting the growth of estrogen-dependent human breast carcinoma Br-10.[13] The results shown in this report indicate that NVB is also effective against hormone-independent breast carcinomas. The expression of mRNA of P-glycoprotein was found to be not detectable in MC-2 and MMKY tumors (unpublished results), and, so far, its correlation with the efficacy of NVB or ADM remains undetermined.

The pattern of plasma concentrations of NVB indicates that it exists in the plasma of nude mice for a relatively long period. Since RIA was used to measure the concentration of NVB, its cross-reactivity with metabolites is a critical matter when discussing the disposition of NVB in plasma. Actually, one possible metabolite, deacetylnavelbine, was reported to react in RIA of NVB,[23] although to a lesser extent than NVB. However, in human patients, metabolites of NVB were hardly detected in plasma and deacetylnavelbine was detected only in urine as a minor metabolites.[26] Therefore, it is concluded that NVB itself exists for a long time in the plasma of human patients. The remarkable antitumor activity of NVB in human patients may be attributed to such plasma disposition and, furthermore, its distribution to various tissues, which was assessed by the large V_{dss} values. The metabolites of NVB in mice are now under investigation.

Conclusion

NVB exhibited growth-inhibitory activity against human breast carcinomas inoculated into nude mice and its correlation with clinical effects was confirmed.

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