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Combination effect of navelbine (vinorelbine ditartrate) with cisplatin against murine P388 leukemia and human lung carcinoma xenografts in mice

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The *in vivo* combination effect of navelbine (NVB, KW-2307) plus cisplatin was compared with that of vindesine (VDS) plus cisplatin in terms of antitumor activity and side effects. The antitumor activity of NVB or cisplatin against i.p. inoculated P388 leukemia was augmented by their combination on various schedules when the interval of administrations was within 24 h. Against i.v. inoculated P388 leukemia, the most significant combination effect was observed when cisplatin was administered 4 h after NVB injection (ILS(%) > 451) and three long-term survivors were observed. On this schedule, the combination of LD_{10} of each drug was achieved, indicating the lack of addition of toxicity. This was further proved by examination of body weight change, white blood cell count and platelet count. Interestingly, significant elevation of blood urea nitrogen concentration by cisplatin was prevented by the combination with NVB. The combination of maximum tolerated dose of NVB and cisplatin was also tolerable in nude mice, and their combination effect was observed against human lung large cell carcinoma Lu-65 and adenocarcinoma PC-12. The number of toxic death mice was more in VDS plus cisplatin-treated groups than in NVB plus cisplatin-treated groups, indicating that the combination chemotherapy of NVB plus cisplatin is a better regimen than that of VDS plus cisplatin in experimental tumor systems.

Key words: Cisplatin, combination, navelbine.

Introduction

Navelbine (NVB, vinorelbine ditartrate, KW-2307) is a new vinca alkaloid analog synthesized by Potier *et al.*[1,2] NVB showed antitumor activity equal or superior to vincristine, vinblastine or vindesine (VDS) in experimental murine and human tumor models including vincristine-resistant murine P388 leukemia.[3–5] 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[6–8] and advanced breast cancer.[9] Among vincristine, vinblastine and VDS, VDS is more effective against solid tumors such as non-small cell lung carcinoma and esophageal carcinoma, and its efficacy is enhanced by combination chemotherapy with cisplatin.[10–14] We demonstrated that NVB induced a synergistic combination effect with cisplatin against human lung adenocarcinoma PC-12 cells *in vitro*.[15] A rationale of this combination chemotherapy was also demonstrated clinically against non-small cell lung cancer and a response rate of 33% was reported.[16] The efficacy of a combination of VDS plus cisplatin[15,17] or NVB plus cisplatin[15] was schedule-dependent *in vitro*. Recently, such schedule dependency of *in vitro* combination effects was also reported between camptothecin, a topoisomerase 1 inhibitor, and etoposide, a topoisomerase II inhibitor.[18]

The present study was carried out in order to examine the *in vivo* combination effect of NVB plus cisplatin on an optimal schedule in terms of antitumor activity and side effects. Furthermore, their combination effect was compared with that of VDS plus cisplatin.

Materials and methods

Chemicals

NVB was provided by Pierre Fabre Médicament (Castre-Cedex, France). VDS was purchased from Shionogi (Osaka, Japan). Cisplatin was purchased from Sigma (St Louis, MO). These compounds were dissolved in sterile 0.9% NaCl solution. The dose of NVB was expressed based on the weight of free base.

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Animals

Male DBA/2Cr mice (6–10 weeks old) and BALB/c × DBA/2Cr F_1 ($CD2F_1$) mice (6–7 weeks old) weighing 22–26 g were obtained from Japan SLC (Shizuoka, Japan). Male BALB/c-*nu/nu* mice (nude mice, 6–7 weeks old) weighing 20–25 g were obtained from Clea Japan (Tokyo, Japan). All animal experiments were conducted with five mice in a group except for those of lethal toxicity.

Antitumor activity

Murine lymphocytic leukemia P388 cells, kindly provided by Dr T Tashiro (Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Tokyo, Japan), were maintained in male DBA/2Cr mice. For the experiments, P388 cells (1×10^6/mouse) were inoculated i.p. or i.v. into $CD2F_1$ mice on day 0. Drug treatment started from day 1 according to the indicated schedule.

Human lung large cell carcinoma LC-6 xenograft was kindly provided by Dr Y Ohnishi (Central Institute for Experimental Animals, Kanagawa, Japan). Xenografts of human lung large cell carcinoma Lu-65 cells, kindly provided by S Hirohashi (National Cancer Center, Tokyo, Japan) and human lung adenocarcinoma PC-12 cells[15] were established by inoculating their cultured cells s.c. into nude mice. Tumor fragments (8 mm^3) were inoculated s.c. into the flanks of nude mice. When the tumor volume was between 100 and 300 mm^3, drugs were injected i.v. 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:[19]

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

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 day of treatment. The T/C value was calculated by the mean V/V_0 value of treated group versus that of control group.

Toxicity

Lethal toxicity was observed for 30 days after drug administration and LD_{10} (10% lethal dose) or LD_{50} (50% lethal dose) values were calculated by the probit method. The body weight of mice was measured by an electronic balance FX-300 (A & D, Tokyo, Japan). For the hematological study, peripheral blood was collected from the retro-orbital sinus of $CD2F_1$ mice. The number of white blood cells (WBC) and platelets was measured by a Toa micro-cell counter CC-180A (Toa Medical Electronics, Hyogo, Japan). Blood urea nitrogen (BUN) was measured by the UN test (Sanko Junyaku, Tokyo, Japan) and Olympus AU510 (Olympus, Tokyo, Japan).

Statistical analysis

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

Results

Schedule dependency of the combination effect of NVB plus cisplatin

We first examined the combination effect of NVB plus cisplatin on various administration schedules in the murine P388 leukemia i.p.–i.p. system (Table 1). The administration of suboptimal dose of NVB (4.5 mg/kg) or cisplatin (6.3 mg/kg) alone increased the life span of i.p. inoculated P388 leukemia-bearing mice (63 and 87%, respectively). Their combination treatment significantly prolonged the life span of tumor-bearing mice, as compared with treatment with NVB or cisplatin alone, on various schedules when the interval of administrations was within 24 h.

Skipper demonstrated that one of the rationales employed for seeking beneficial combination chemotherapy was to detect combinations which were less than additive with respect to host toxicity.[20] Therefore we further examined the rationales of combination chemotherapy of NVB plus cisplatin or VDS plus cisplatin with respect to lethal toxicity. The LD_{10} values of NVB, VDS and cisplatin in $CD2F_1$ mice were 16.2, 5.6 and 11.4 mg/kg, respectively, by single i.v. injection (Table 2). Based on these LD_{10} values, the combination effect of NVB plus cisplatin was examined at various doses against i.v. inoculated P388 leukemia (Table 3). The maximum effect of NVB or cisplatin used alone was observed at their LD_{10}. When NVB and cisplatin were used in combination, the degree of maximum effect varied

Table 1. Schedule dependency of combination effect of NVB plus cisplatin against i.p. inoculated murine P388 leukemia

Administration schedule				Mean survival days ± SD	ILS (%)	>30 days survivors
0 h	4 h	8 h	24 h			
–	—	—		10.4 ± 0.6	0	0/5
NVB	—	—		17.0 ± 0.7[a]	63	0/5
Cisplatin	—	—		19.4 ± 2.2[a]	87	0/5
NVB + cisplatin	—	—		>26.6 ± 4.1[a,b]	>156	2/5
NVB	cisplatin	—		>24.4 ± 3.4[a,b]	>135	1/5
NVB	—	cisplatin		>25.4 ± 3.1[a,b]	>144	1/5
Cisplatin	NVB	—		24.4 ± 1.3[a,b]	135	0/5
Cisplatin	—	NVB		26.8 ± 2.6[a,b]	158	0/5
—			—	10.8 ± 0.4	0	0/5
NVB			—	16.2 ± 2.6[a]	50	0/5
—			cisplatin	20.0 ± 2.3[a]	85	0/5
NVB			cisplatin	>28.0 ± 2.3[a,b]	>159	1/5
Cisplatin			—	16.0 ± 1.0[a]	48	0/5
—			NVB	17.6 ± 2.2[a]	63	0/5
Cisplatin			NVB	>23.2 ± 5.0[a,b]	>115	1/5

P388 cells (1×10^6/mouse) were inoculated i.p. on day 0. NVB (4.5 mg/kg) or cisplatin (6.3 mg/kg) was administered i.p. on day 1 or 2 following the indicated schedule.
[a] ILS ≥ 25 (%) and $p < 0.05$ versus untreated group.
[b] $p < 0.05$ versus NVB-treated group and cisplatin-treated group respectively, on corresponding schedules.

Table 2. Lethal toxicity of NVB, VDS and cisplatin in $CD2F_1$ mice

Drugs	Dose (mg/kg)	Mortality	MTD (mg/kg)	LD_{10} (mg/kg)	LD_{50} (mg/kg)
NVB	13.9	0/10	13.9	16.2	20.2
	16.7	1/10			
	20.0	5/10			
	24.0	10/10			
	28.8	9/10			
	34.6	10/10			
VDS	4.02	0/10	4.0	5.6	7.3
	4.82	1/10			
	5.79	0/10			
	6.94	5/10			
	8.33	6/10			
	10.0	10/10			
Cisplatin	7.68	0/8	7.7	11.4	16.3
	11.5	1/8			
	17.3	4/8			
	25.9	8/8			

NVB, VDS or cisplatin was administered i.v.

depending on their treatment schedule. The most significant combination effect was observed when cisplatin was injected 4 h after NVB injection. The increase in life span (ILS) was over 451% and three long-term survivors were observed. In this case, the combination of LD_{10} (NVB, 16.2 mg/kg; cisplatin, 11.4 mg/kg) was achieved. On the other hand, when NVB was injected simultaneously with cisplatin or 4 h after cisplatin injection, the combination effect decreased to some extent.

Lethal toxicity of combination chemotherapy

Since the sequential treatment of NVB and cisplatin at 4 h intervals was recommended from the above result, the lethal toxicity of combination therapy of NVB plus cisplatin or VDS plus cisplatin was examined on this schedule (Table 4). Maximum tolerated dose (MTD) of cisplatin (7.7 mg/kg) was administered 4 h after NVB or VDS administration, and LD_{10} values of NVB and VDS were compared. Interestingly the LD_{10} values of NVB (17.3 mg/kg) or VDS (5.8 mg/kg) did not decrease even after the combination with cisplatin (7.7 mg/kg), indicating that the combination of NVB or VDS with cisplatin was a rational therapy because of the lack of addition of toxicity.

Antitumor activity against human non-small cell lung carcinomas

Based on these results, the efficacy of the combination of NVB plus cisplatin was compared with that of VDS plus cisplatin against human non-small cell lung carcinomas inoculated into nude mice (Table 5 and Figure 1). In this experiment,

Table 3. Combination effect of NVB plus cisplatin against i.v. inoculated murine P388 leukemia

Administration schedule		Dose (mg/kg)		Mean survival days ± SD	ILS (%)	>60 days survivors
0 h	4 h	NVB	Cisplatin			
—	—	0	0	7.4 ± 0.5	0	0/5
NVB	—	4.8	0	11.2 ± 0.4[a]	51	0/5
		7.2	0	12.2 ± 0.4[a]	65	0/5
		10.8	0	14.2 ± 1.1[a]	92	0/5
		16.2	0	16.6 ± 1.7[a]	124	0/5
Cisplatin	—	0	3.4	12.8 ± 1.1[a]	73	0/5
		0	5.1	14.4 ± 0.5[a]	95	0/5
		0	7.6	17.0 ± 1.6[a]	130	0/5
		0	11.4	21.6 ± 11.5[a]	192	0/5
NVB + cisplatin	—	4.8	3.4	22.0 ± 2.3[a,b]	197	0/5
		7.2	5.1	21.0 ± 3.4[a,b]	184	0/5
		10.8	7.6	27.0 ± 6.7[a,b]	265	0/5
		16.2	11.4	>16.0 ± 24.5[a]	>116	1/5
NVB	cisplatin	4.8	3.4	14.8 ± 0.8[a,b]	100	0/5
		7.2	5.1	20.0 ± 2.9[a,b]	170	0/5
		10.8	7.6	27.0 ± 1.9[a,b]	265	0/5
		16.2	11.4	>40.8 ± 27.0[a]	>451	3/5
Cisplatin	NVB	4.8	3.4	14.8 ± 2.9[a]	100	0/5
		7.2	5.1	18.3 ± 1.0[a,b]	147	0/5
		10.8	7.6	24.2 ± 4.3[a,b]	227	0/5
		16.2	11.4	>21.6 ± 25.9[a]	>192	1/5

P388 cells (1×10^6/mouse) were inoculated i.v. on day 0. Drugs were administered i.v. on day 1 following the indicated schedule.
[a] ILS ≥ 25 (%) and $p < 0.05$ versus untreated group.
[b] $p < 0.05$ versus NVB-treated group and cisplatin-treated group respectively, at corresponding doses.

Table 4. Influence of cisplatin on lethal toxicity of NVB or VDS in $CD2F_1$ mice

Drugs	Dose (mg/kg)	Cisplatin			
		0 mg/kg		7.7 mg/kg	
		mortality	LD_{10} (mg/kg)	mortality	LD_{10} (mg/kg)
Control	0	0/18		0/18	
NVB	13.9	0/18	17.3	0/18	23.9
	16.7	1/18		1/18	
	20.0	5/18		0/18	
	24.0	10/18		1/18	
	28.8	11/18		3/18	
	34.6	14/18		15/18	
	41.5	18/18		18/18	
VDS	4.02	0/18	5.8	0/18	5.8
	4.82	1/18		0/18	
	5.79	0/18		2/18	
	6.94	8/18		8/18	
	8.33	13/18		13/18	
	10.0	18/18		18/18	

NVB or VDS was administered i.v. After 4 h, the MTD of cisplatin (7.7 mg/kg) was administered i.v.

MTD of NVB or VDS in nude mice[5] was administered in combination with the LD_{10} of cisplatin at the 4 h interval. These combinations were also tolerated in nude mice and the accumulation of toxicity was insignificant (Table 5). The augmentation of antitumor activity by combination was observed against Lu-65 and PC-12, which were less sensitive to each drug, suggesting the rationale of these combination chemotherapies. With respect to the comparison of NVB and VDS, the antitumor activity of VDS used alone was less significant than that of NVB against LC-6 and Lu-65. Furthermore, the number of toxic death mice was more in VDS plus cisplatin-treated groups than in NVB plus cisplatin-treated groups, suggesting that the combination of NVB plus cisplatin is a better regimen than that of VDS plus cisplatin.

Side effects of combination chemotherapy

To examine the usefulness of the combination further, body weight change, BUN concentration,

Table 5. Combination effect of NVB plus cisplatin or VDS plus cisplatin against human non-small cell lung carcinomas inoculated into nude mice

Drugs	LC-6 large cell			Lu-65 large cell			PC-12 adenocarcinoma		
	T/C (%)	BW[a] (g)	mortality	T/C (%)	BW (g)	mortality	T/C (%)	BW (g)	mortality
NVB	6[b]	−3.3	0/5	29[b]	−0.7	0/5	82	−1.6	0/5
VDS	14[b]	−3.7	0/5	39[b]	−2.4	0/5	80	−3.0	0/5
Cisplatin	15[b]	−4.2	0/5	35[b]	−3.2	0/5	75	−4.6	0/5
NVB + cisplatin	7[b]	−6.0	0/5	19[b,c]	−5.0	1/5	40[b,c]	−3.3	0/5
VDS + cisplatin	10[b]	−5.7	1/5	15[b,c]	−7.4	2/5	46[b,c]	−6.4	1/5

MTD of NVB (16.2 mg/kg) or VDS (3.3 mg/kg) was administered i.v. into tumor-bearing nude mice. After 4 h, cisplatin (11.0 mg/kg) was administered i.v. Survival days were observed for 14 days.
[a] Maximum body weight change.
[b] T/C(%) ≤ 50 and $p < 0.01$ (one-sided) versus untreated group.
[c] $p < 0.05$ versus NVB- or VDS-treated group and cisplatin-treated group respectively.

WBC and platelet number were measured in $CD2F_1$ mice (Figure 2). The body weight decrease was more significant in cisplatin-treated mice than in NVB- or VDS-treated mice. However, its accumulation was not indicated by the combination of NVB plus cisplatin or VDS plus cisplatin. The decrease of WBC number or platelet number was more significant in NVB- or VDS-treated mice than in cisplatin-treated mice. The accumulation of these side effects was also not induced by both combination regimens. Interestingly, the significant elevation of BUN concentration in cisplatin-treated mice, which was supposed to be associated with its renal toxicity, was not observed in mice pretreated with NVB or VDS.

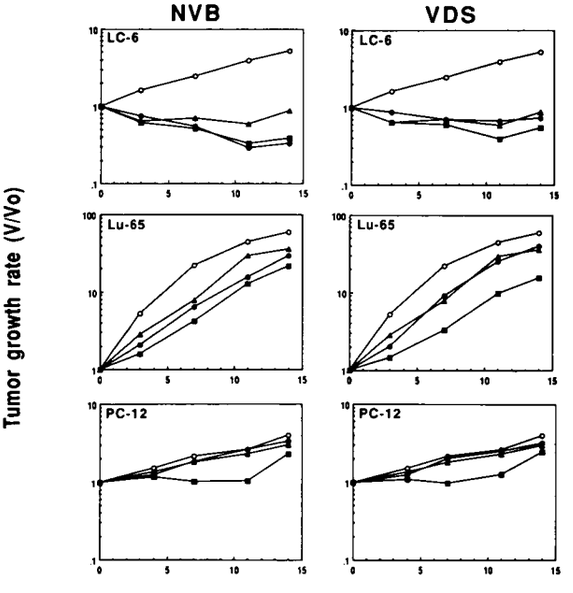

Figure 1. Growth pattern of human non-small cell lung carcinomas treated with NVB plus cisplatin or VDS plus cisplatin. Human lung large cell carcinoma LC-6, Lu-65 and adenocarcinoma PC-12 were treated as described in Table 5: ○, untreated, ●, NVB or VDS; ▲, cisplatin; ■, combination.

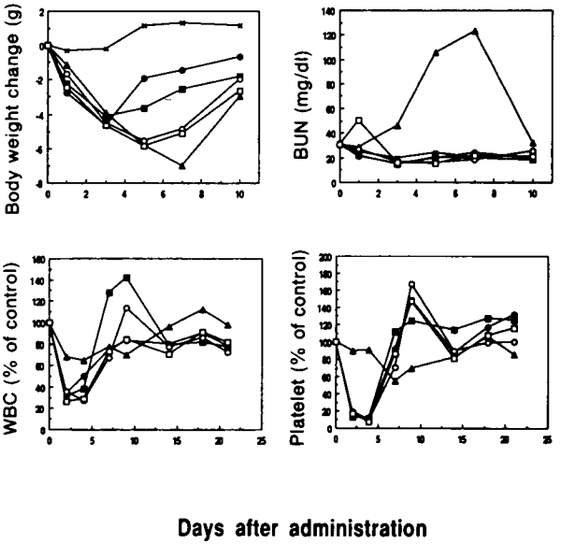

Figure 2. Side effects of combination therapy of NVB plus cisplatin or VDS plus cisplatin in $CD2F_1$ mice. LD_{10} of KW-2307 (16.2 mg/kg) or VDS (5.6 mg/kg) was administered i.v. and, after 4 h, cisplatin (11.4 mg/kg) was administered i.v.: ×, untreated; ●, NVB; ■, VDS; ▲, cisplatin; ○, NVB plus cisplatin; □, VDS plus cisplatin.

Discussion

To achieve successful combination cancer chemotherapy, Skipper demonstrated three basic categories:[20]

(i) Combinations of certain drugs which lessen the emergence of true drug resistance.
(ii) Combinations of drugs which are less than additive in host toxicity.
(iii) Combinations of drugs which possess different biochemical–cytokinetic characteristics.

As for the combination chemotherapy of NVB plus cisplatin, we previously found the *in vitro* synergistic cytotoxicity by their sequential treatment using human lung adenocarcinoma PC-12 cells and showed that this combination effect may be due to their different cytokinetic characteristics by analyzing the cell cycle distribution of PC-12 cells.[15] This result indicates that the combination of NVB plus cisplatin agrees with the third category *in vitro*. The present study was conducted to examine the *in vivo* combination effect of NVB plus cisplatin on an optimal schedule.

Against i.v. inoculated P388 leukemia, the most significant combination effect was observed when cisplatin was injected 4 h after NVB injection (ILS(%) > 451) and three long-term survivors were observed (Table 3). On this schedule, the combination of LD_{10} (NVB, 16.2 mg/kg; cisplatin, 11.4 mg/kg) was achieved, indicating the lack of addition of toxicity. This was proved by the examination of lethal toxicity, body weight decrease, WBC count and platelet count in normal $CD2F_1$ mice (Table 4 and Figure 2). The combination of the MTD of NVB and cisplatin was also tolerable in nude mice, and produced the augmentation of their antitumor effect against human lung large cell carcinoma Lu-65 and adenocarcinoma PC-12, which were less sensitive to each drug (Table 5 and Figure 1). These results indicate that the *in vivo* combination of NVB plus cisplatin also agrees with the second concept.

We previously demonstrated that antagonism was observed *in vitro* when NVB or VDS was used in combination with cisplatin simultaneously for 24 h.[15] Such antagonism between vinca alkaloids and cisplatin in the case of simultaneous exposure has already been reported.[17] However in *in vivo* i.p. inoculated P388 leukemia-bearing mice, the synergistic combination effect of NVB plus cisplatin was observed in various combination schedules when the interval of administration was within 24 h (Table 1). This result indicates that the schedule dependency of *in vivo* combination effect is not necessarily inconsistent with *in vitro* schedule dependency, although the reason for this still remains undetermined.

We also reported that the MTD of NVB in nude mice was about 4.8 times more than that of VDS and this might be associated with the superior antitumor activity of NVB to VDS.[5] In this study, a total of four toxic death mice were observed in the VDS plus cisplatin-treated groups, whereas only one toxic death mouse was observed in the NVB plus cisplatin-treated groups (Table 5). These results indicate that combination chemotherapy with NVB plus cisplatin is a better regimen than that of VDS plus cisplatin in experimental tumor systems.

Conclusion

The combination chemotherapy of NVB plus cisplatin is rational in terms of not only biochemical cytokinetic characteristics but also in terms of host toxicity.

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