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Positive interactions between human interferon and cepharanthin against human cancer cells in vitro and in vivo

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Abstract A human tumor microcytotoxicity-viable cell-staining assay was used to test the antiproliferative effect of recombinant human interferon-beta or -gamma alone and in combination with bisbenzylisoquinoline alkaloid cepharanthin against four human tumor cell lines in vitro and in nude mice. Results obtained in the in vitro study indicate that combinations of interferon-beta/-gamma with cepharanthin show synergistic and, occasionally, additive antiproliferative effects in a dose-dependent manner on tumor viable cell-staining assay. Interferon-gamma combined with cepharanthin suppressed the growth of all four human tumor cell lines (RPMI 4788, PC 10, HeLa, ZR-75-1), and this enhanced antiproliferative effect was not dependent on the interferon species involved, including interferon-beta and -gamma. In an experimental model of pulmonary metastasis, in which human colon tumor cells were inoculated i.v. into nude mice, interferon-gamma alone exerted significant inhibitory activity against pulmonary metastasis in a dose-dependent manner, and cepharanthin alone also significantly inhibited metastasis. Furthermore, a combination of interferon-gamma with cepharanthin resulted in a considerable suppression of pulmonary metastasis. These studies indicate that due to their therapeutic potential, combinations of recombinant human interferon-beta or -gamma with cepharanthin might be a promising therapy for pulmonary metastasis of human cancers.

Key words Interferon · Cepharanthin · Antitumor effect

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Introduction

It is now well established that interferon (IFN) has antitumor activity in both animals and humans, but IFN therapy alone is not sufficient to cause complete remission of established disease in many tumors [1–4]. Although IFN is an immune-modifying agent, its antitumor activity is primarily evaluated in current clinical trials. Knowledge that IFN induces many cellular physiological changes associated with its antiproliferative activity is very important for the effective use if IFN in cancer therapy, especially in its combination therapy with other chemotherapeutic agents or biological response modifiers (BRMs) having different action mechanisms, since the use of IFN alone produces only limited activity. If a combination of IFN and a chemotherapeutic agent could broaden the antitumor spectrum of the chemotherapeutic agent, it might permit a reduction in the dose of the chemotherapeutic agent. In fact, many investigators have reported synergistic interactions between human IFN and cytotoxic drugs [5, 6]. In addition, in vitro studies suggest that combinations of IFN with various chemotherapeutic agents may enhance cytotoxicity for tumor cells [7–10].

Cepharanthin is a drug consisting of bisbenzylisoquinoline (biscoclaurine) alkaloids extracted from *Stephania cepharantha* HAYATA and has been used widely in the treatment of diseases such as leukopenia [11], nasal allergy [12], and snake-venom injuries [13], among others, in Japan. Cepharanthin is a membrane-interactive agent that has membrane-stabilizing activity influencing the fluidity of the lipid bilayer in the cell membrane [14]. This drug also alters the transmembrane movement of Ca^{2+} [15–17] and K^+ [14, 18]. The membrane-stabilizing action of cepharanthin is effective in potentiating the cytotoxic activity of anticancer drugs [16, 17, 19, 20]. Furthermore, cepharanthin exerts immunomodulatory effects by enhancing the cytotoxic effect of natural killer cells [21] and macrophages [22].

On the basis of this background, we examined the therapeutic potential of combinations of two kinds of re-

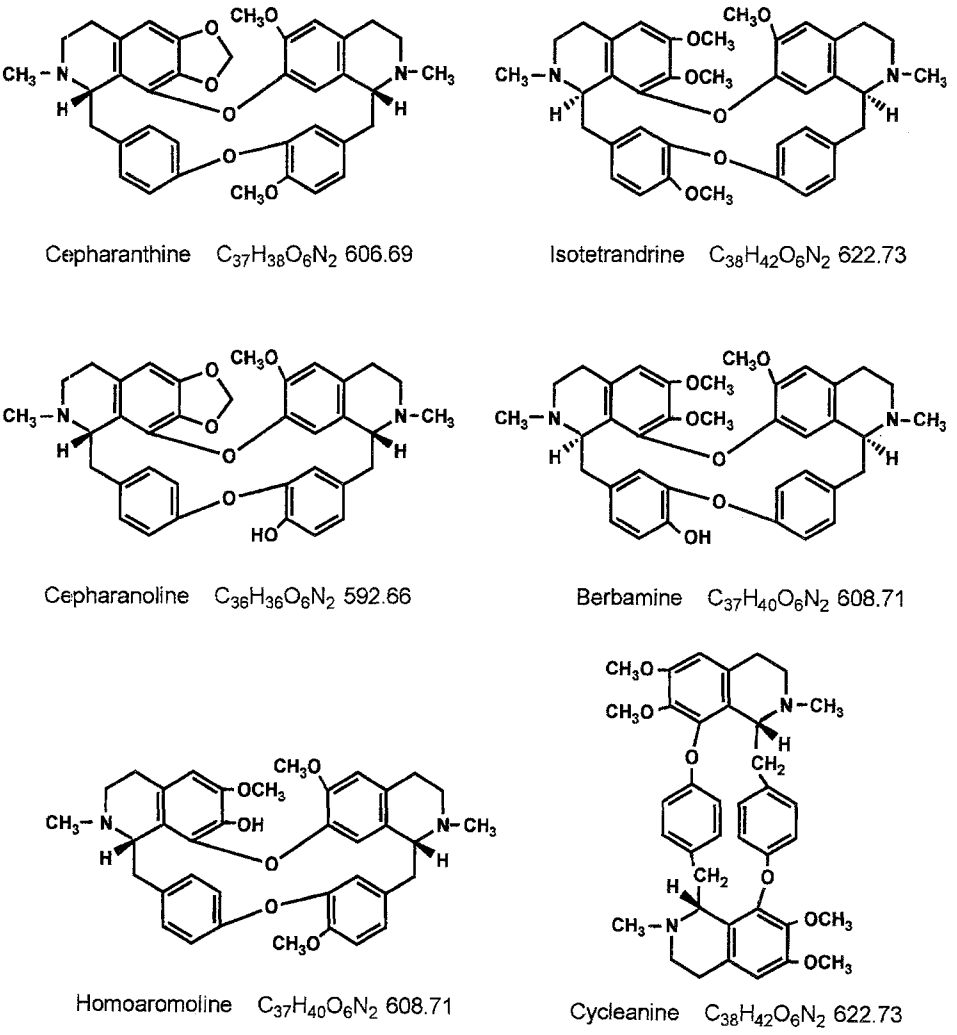

Fig. 1 Structures of six bis-benzylisoquinoline alkaloids in cepharanthin

combinant human IFN (rIFN-β or rIFN-γ) with cepharanthin, in place of a chemotherapeutic agent, in a human tumor model system in vitro and in vivo.

Materials and methods

Mice

Male BALB/c (nu/nu) mice were purchased from SLC Co. (Hamamatsu, Shizuoka, Japan) and used for experiments at the age of 6–8 weeks. Mice were maintained under specific pathogen-free conditions using laminar flow racks and were fed sterile food and water in our experimental-animal room.

Tumor cells

Cell lines used for drug assays were derived from a human colon cancer (RPMI 4788) [23], a human lung carcinoma (PC 10) [24], a human uterine cervical cancer (HeLa) [25], and a human breast cancer (ZR-75-1) [26]. All were serially maintained as monolayer cultures fed with RPMI 1640 medium plus 10% heat-inactivated fetal calf serum (FCS) supplemented with penicillin (100 IU/ml), streptomycin (100 μg/ml), L-glutamine (2 m*M*), and 25 m*M* *N'*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (Gibco, Grand Island, N.Y., USA) at 37 °C under a humidified atmosphere containing 5% CO_2. Cells in the exponentially proliferating phase were harvested for the experiments.

Interferons and drug

Recombinant human interferon-β (rIFN-β) [27, 28] prepared by *Escherichia coli* recombinant DNA technology was provided by Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan). This preparation is more than 99.5% pure and has a specific activity of over 5×10^7 IU/mg protein. Recombinant human interferon-γ (rIFN-γ) [29] produced by *E. coli* recombinant DNA technology (Kyowa Hakko Kogyo, Co., Ltd., Tokyo) has a purity of over 99% and a specific activity of over 5×10^6 Japanese reference units (JRU)/mg protein. The antiviral activity was assayed in terms of the cytopathic effect of vesicular stomatitis virus on FL cells and was expressed with reference to the National Institutes of Health (USA) standard (GO23-902-527). The titer of rIFN-γ was expressed in Japanese reference units per milliliter according to the Japanese rIFN-γ reference, which had been established on the basis of the international units of the WHO IFN-γ reference (Gg23-901-530). Cepharanthin, a drug consisting of bisbenzylisoquinoline (biscoclaurine) alkaloids, was donated by Kaken Shoyaku Co., Ltd., Tokyo. The chemical structures of these alkaloids are shown in Fig. 1. These IFNs and cepharanthin were freshly diluted to the appropriate dose at each time point in growth medium for in vitro experiments and in physiological saline for in vivo experiments.

Human tumor microcytotoxicity-viable cell-staining assay

The microcytotoxicity assay was used as previously described by Klein [30]. The following modifications were introduced, adapting the technique to the simple growth requirements of the cell lines. Viable tumor cells were plated in 96-well microtest flat plates (Falcon Lab-

ware, Oxnard, Calif., USA) at a concentration of 1×10^4 cells/well. Cells were subcultured in 0.2 ml of RPMI 1640 medium with 10% FCS. After a 24-h period of preincubation at 37 °C in an incubator containing 5% CO_2, the medium was aspirated. Then, cepharanthin (0.1 μg/ml), rIFN-β (10^4 or 10^5 IU/ml) and rIFN-γ (10^4 or 10^5 JRU/ml) diluted in 0.2 ml of RPMI 1640 with 10% FCS were added, alone or simultaneously in combination, and cultures were incubated for 2 days at 37 °C in an incubator containing 5% CO_2. The rIFN-β-, rIFN-γ-, or cepharanthin-containing medium and the growth medium for the control culture were changed daily. After 2 days of incubation, the medium was aspirated, cells were washed twice with medium, and then the surviving cells were fixed with methanol and stained with 0.5% crystal violet [31]. The plates were rinsed twice with phosphate-buffered saline (PBS) and air-dried overnight.

The stained cells were solubilized with Sorenson's buffer (0.1 *M* glycine and HCl, pH 3.0) in 30% ethanol and the absorbance of each well was measured at 450-nm/600-nm wavelengths with a multiscan spectrophotometer, Micro Plate Reader model MPR A4 (Toso Co., Tokyo). The absorbance of the eluted dye was found to be linearly correlated with the number of viable cells [Ono et al., submitted for publication]. Repeated experiments indicated that good reproducibility was obtained with this dye-uptake method. The mean absorbance of four determinations was employed to calculate the index of cell growth. The index of cell growth was obtained according to the following formula [33]:

$$\text{Index (\% of control)} = \frac{\text{mean absorbance of the treated group}}{\text{mean absorbance of the control group}} \times 100.$$

Definitions of combined effect by the microcytotoxicity-viable cell-staining method

The mean and standard deviation (SD) of the index were calculated and the mean index value obtained for the wells treated with a combination of cepharanthin and rIFN-γ or rIFN-β was compared with the expected value calculated by multiplication of the mean index values obtained for the wells treated with each agent alone. If the difference between the experimental value and the expected value was more than 2SD or less than -2DS, we judged the combined effect to be synergistic or antagonistic, respectively. If the difference ranged from -2SD to 2SD, we judged the combined effect to be additive [33].

Evaluation of the antitumor effect of interferon and cepharanthin in combination against experimental pulmonary metastasis in nude mice

For the establishment of pulmonary metastasis, each BALB/c (nu/nu) mouse was given 2×10^6 cells of a single-cell suspension of RPMI 4788 cells in a 0.1-ml vol. by injection into the tail vein using a 27-gauge needle (day 0). Histological examination carried out 2 days after the injection of tumor cells revealed established micrometastasis in the lung [34].

From day 2 through day 11, mice were given rIFN-γ (1×10^5 or 5×10^5 JRU/mouse per 0.1 ml) and cepharanthin (5 mg/kg per 0.1 ml), alone or in combination, by injection into the tail vein. Physiological saline (0.1 ml/mouse) was injected i.v. beginning on day 2 for 10 consecutive days in the control group. Six mice were included in each treated group. At 21 days after tumor inoculation, the mice were euthanized for enumeration of metastatic pulmonary nodules. Metastatic pulmonary nodules were counted in a blind fashion without knowledge of the treatment given a mouse according to the method of Wexler [35]. The percentage of inhibition induced by each treatment was calculated by means of the following formula:

$$\text{\% Inhibition} = \left(1 - \frac{\text{metastatic nodules of the experimental group}}{\text{metastatic nodules of the control group}}\right) \times 100.$$

The significance of differences in the numbers of lung metastases detected between groups was determined using Student's *t*-test.

Results

Antiproliferative activity of rIFNs and cepharanthin, alone or in combination, against RPMI 4788 cells in vitro

The level of antiproliferative activity was generally dependent on the concentration of rIFN-γ or rIFN-β, although the activity was weak at low concentrations. Antiproliferative activities of rIFN-γ or rIFN-β were augmented in an additive or a synergistic manner by cepharanthin. For example, rIFN-γ plus a dose of cepharanthin (0.1, 1, or 5 μg/ml) showed a synergistic effect, and rIFN-γ plus a dose of 0.01 μg/ml cepharanthin produced an additive effect. A high-dose combination of rIFN-γ (1×10^4 JRU/ml) and cepharanthin (5 μg/ml) gave the most potent antiproliferative activity, which was 74.9% {(1-16.6/66.2)×100} more than the expected value (Table 1). Also, the combined effect induced by rIFN-β and cepharanthin was found to be similar to the effect produced by the combination of rIFN-γ with cepharanthin (Table 2). Since the activity increased with increasing concentrations of both rIFNs and cepharanthin, these findings support the interpretation that antiproliferative activity is the result of a common synergistic event by which rIFNs and cepharanthin potentiate each other. The antiproliferative activities

Table 1 Combined effects of rIFN-γ and cepharanthin on human colon RPMI 4788 cell proliferation[a]

rIFN-γ concentration (JRU/ml)	Cepharanthin concentration (μg/ml)				
	0	0.01	0.1	1	5
0	100.0 ± 2.7	99.5 ± 3.7	98.9 ± 4.4	97.5 ± 5.0	92.2 ± 2.0
2×10^2	88.9 ± 1.1	84.1 ± 3.7[b] (88.5)	83.0 ± 4.7[b] (87.9)	76.3 ± 4.6[c] (86.7)	69.1 ± 4.2[c] (82.0)
1×10^3	85.8 ± 3.3	82.2 ± 2.0[b] (85.4)	70.2 ± 6.1[c] (84.9)	67.0 ± 4.7[c] (83.7)	44.2 ± 3.4[c] (79.1)
5×10^3	82.5 ± 5.7	77.9 ± 4.1[b] (82.1)	66.0 ± 4.8[c] (81.6)	52.3 ± 3.9[c] (80.4)	30.4 ± 3.9[c] (76.1)
1×10^4	71.8 ± 4.5	65.2 ± 2.6[c] (71.4)	48.3 ± 3.8[c] (71.0)	36.2 ± 5.7[c] (70.0)	16.6 ± 2.8[c] (66.2)

[a] RPMI 4788 cells (1×10^4/well) were cultivated in vitro in a microtest plate for 2 days in the presence of rIFN-γ and/or cepharanthin. The indices for the percentage of control values (mean ± SD for quadruplicate determinations) at each combination of concentrations are shown. Data in parentheses are the values expected if the two agents were additively effective. For calculation of expected values, see Materials and methods

[b] Additive effect

[c] Synergistic effect

Table 2 Combined effects of rIFN-β and cepharanthin on human colon RPMI 4788 cell proliferation[a]

rIFN-β concentration (IU/ml)	Cepharanthin concentration (μg/ml)				
	0	0.01	0.1	1	5
0	100.0 ± 2.6	99.5 ± 3.7	98.9 ± 4.4	97.5 ± 5.0	92.2 ± 2.0
2×10^2	98.5 ± 2.3	93.1 ± 3.6[b] (98.0)	92.5 ± 5.5[b] (97.4)	86.9 ± 4.7[b] (96.0)	76.5 ± 3.6[c] (90.8)
1×10^3	96.4 ± 3.0	92.1 ± 3.2[b] (95.9)	81.0 ± 3.3[c] (95.3)	79.9 ± 3.7[c] (94.0)	51.8 ± 3.7[c] (88.9)
5×10^3	90.1 ± 3.2	85.1 ± 4.2[b] (89.6)	78.4 ± 3.9[c] (89.1)	70.2 ± 4.1[c] (87.8)	33.2 ± 4.8[c] (83.1)
5×10^4	75.2 ± 2.2	72.6 ± 3.0[b] (74.8)	64.7 ± 2.8[c] (74.4)	43.9 ± 3.8[c] (73.3)	25.9 ± 2.1[c] (69.3)

[a] The indices for the percentage of control values (mean ± SD for quadruplicate determinations) at each combination of concentrations are shown. Data in parentheses are the values expected if the two agents were additively effective. For calculation of expected values, see Materials and methods; for details, see the footnote to Table 1
[b] Additive effect
[c] Synergistic effect

Table 3 IFN species in enhanced suppression of PC 10 cell proliferation in combination with cepharanthin[a]

IFN species		% Control cell survival[b] in the presence of cepharanthin (μg/ml)		Expected values[c]	Evaluation[d]
		0	5		
Control (medium)		100.0 ± 3.2	88.6 ± 4.2		
rIFN-β	10^3 IU/ml	92.3 ± 3.8	56.7 ± 2.9	81.8	Synergistic
	10^4 IU/ml	68.7 ± 5.5	37.8 ± 2.7	60.9	Synergistic
rIFN-γ	10^3 JRU/ml	82.5 ± 3.5	46.8 ± 4.7	73.1	Synergistic
	10^4 JRU/ml	51.3 ± 3.7	29.0 ± 4.9	45.5	Synergistic

[a] PC 10 cells (1×10^4/well) were cultivated in vitro in a microtest plate for 2 days in the presence of interferons (rIFN-β, rIFN-γ) and cepharanthin, alone or in combination
[b] Mean values ± SD for quadruplicate determinations
[c] Values expected if the two agents were additively effective
[d] For details on the evaluation of the combination of IFN and cepharanthin, see Materials and methods

of the combinations of rIFN-γ (2×10^2 JRU/ml) or rIFN-β (2×10^2 IU/ml) with cepharanthin (5 μg/ml) were almost equivalent to the effect of a higher concentration of rIFN-γ (1×10^4 JRU/ml) alone and rIFN-β (5×10^4 IU/ml) alone, respectively (Tables 1, 2).

IFN species dependency of the enhanced suppression of cell proliferation

rIFN-β and rIFN-γ were tested for their ability to produce enhanced suppression of the proliferation of human lung carcinoma PC 10 cells in combination with cepharanthin (Table 3). The results showed that enhanced suppression was achieved by each of the rIFNs with cepharanthin.

Cell selectivity of the enhanced antiproliferative activity

The cell selectivity of the enhanced suppression induced by rIFN-γ and cepharanthin was tested (Table 4). When four different cell types were cultivated under conditions such that similar extents of cell proliferation were expected, enhanced antiproliferative activity was achieved with all the cell types, i.e., RPMI 4788, PC 10, HeLa, and ZR-75-1 cells. These results indicated that in the presence of rIFN-γ and cepharanthin, these four cell types responded in a similar fashion.

Effect of rIFN-γ and cepharanthin on pulmonary metastasis

As shown in Table 5, rIFN-γ and cepharanthin individually exerted significant inhibitory effects against pulmonary metastasis as compared with the control group (pulmonary metastatic nodules, 348.0 ± 33.2). Therefore, the inhibition rates were shown to be 31.3% for the group treated with rIFN-γ at 1×10^5 JRU/mouse, 59.6% for the group treated with rIFN-γ at 5×10^5 JRU/mouse, and 28.1% for the group treated with cepharanthin (5 mg/kg) as compared with the control group. When rIFN-γ at 5×10^5 JRU/mouse was combined with cepharanthin, the inhibitory effect was augmented synergistically. There were 153.0 ± 24.9 and 58.8 ± 20.3 pulmonary metastatic nodules in the group treated with cepharanthin plus rIFN-γ at 1×10^5 JRU/mouse and the group treated with rIFN-γ at 5×10^5 JRU/mouse (one of six mice were completely cured), respectively ($P < 0.001$), exhibiting that their numbers were strongly reduced in these groups as compared with the control group.

Table 4 Cell selectivity of enhanced suppression of cell proliferation by rIFN-γ and cepharanthin[a]

Agents added	% Control cell concentration[b] of			
	RPMI 4788	PC 10	HeLa	ZR-75-1
rIFN-γ[c]	82.5 ± 5.7	78.7 ± 3.9	89.7 ± 3.8	87.5 ± 4.5
Cepharanthin[d]	92.2 ± 2.0	88.6 ± 4.2	97.5 ± 2.2	92.8 ± 2.4
rIFN-γ + cepharanthin	30.4 ± 3.9[e] (76.1)	46.0 ± 2.7[e] (69.7)	69.1 ± 2.5[e] (87.5)	47.9 ± 5.4[e] (81.2)

[a] RPMI 4788, PC 10, HeLa, and ZR-75-1 cells (1×10^4/well) were cultivated in vitro for 2 days in the presence of rIFN-γ and/or cepharanthin
[b] Mean values ± SD for quadruplicate determinations
[c] 5×10^3 JRU/ml
[d] 5 μg/ml
[e] Values deviating from the expected values (in parentheses) by synergistic effect

Table 5 Inhibitory effect of rIFN-γ and/or cepharanthin on experimental pulmonary metastases in nude mice

Treatment[a]	Number of metastatic nodules[b] (mean ± SD)	% Inhibition[c]	Incidence of pulmonary metastasis
Control (saline)	348.0 ± 33.2		6/6
rIFN-γ 1×10^5 JRU	239.1 ± 44.5*	31.3	6/6
rIFN-γ 5×10^5 JRU	140.6 ± 33.0**	59.6	6/6
Cepharanthin 5 mg/kg	250.2 ± 31.2*	28.1	6/6
rIFN-γ 1×10^5 JRU + cepharanthin	153.0 ± 24.9**	56.0	6/6
rIFN-γ 5×10^5 JRU + cepharanthin	58.8 ± 20.3**	83.1	5/6

* $P < 0.01$ vs control; ** $P < 0.001$ vs control
[a] BALB/c nude mice were injected i.v. with 2×10^6 RPMI 4788 cells/mouse (day 0), and then rIFN-γ (1×10^5 or 5×10^5 JRU/mouse) and cepharanthin (5 mg/kg) were given i.v. beginning on day 2 for 10 consecutive days, alone or in combination
[b] On day 21, the mice were euthanized and the numbers of pulmonary metastatic nodules were counted according to the method of Wexler [35]
[c] % Inhibition = (1 –experimental metastatic nodules/control metastatic nodules) × 100

Discussion

The present study demonstrated that the in vitro and in vivo antitumor effects of rIFN-γ or rIFN-β against four different human tumor cell lines, i.e., RPMI 4788 colon cancer, PC 10 lung carcinoma, HeLa uterine cervical cancer, and ZR-75-1 mammary cancer, were augmented by their combination with cepharanthin to bring about additive to synergistic effects. Several investigators have observed that the activity of some anticancer agents is enhanced when they are combined with cepharanthin in vitro [16, 17, 36, 37], and positive interactions have been confirmed in several murine tumor models as well [16]. In the present in vitro experiments, additive effects of rIFN-γ and cepharanthin as well as rIFN-β and cepharanthin were observed at low-dose combinations and synergistic effects were found at high-dose combinations. A more than additive antiproliferative effect in RPMI 4788 cells was shown for combinations of rIFN-γ with cepharanthin. These results indicate that the mutual interaction between cepharanthin and rIFN-γ or rIFN-β is dose-dependent in vitro. Thus, we conclude that the interactions between IFN and cepharanthin are positive or synergistic. Also, unequivocal evidence of a positive interaction can be obtained when one of the agents in the combination has little effect, as can be seen in Tables 1 and 2, where the dose of cepharanthin (5 μg/ml) has no effect on the tumor-cell proliferation index but strongly increases the effect of IFN when the two substances are used in combination.

We examined the factors involved in this enhancement and obtained the following findings. The enhancement had no connection with the IFN species involved, although the cells' sensitivity to different IFNs was variable. Suppression of PC 10 cell proliferation by rIFN-γ plus cepharanthin was more enhanced than that induced by rIFN-β in combination with cepharanthin (Table 3). Also, the enhancement was not cell-selective, as it was achieved in four different cell types by the combination of rIFN-γ with cepharanthin. These findings indicate that the cells' sensitivity to the agents used is not the only factor involved in this enhancement and that the IFN and cepharanthin combinations interact with cells in the presence and the absence of induction of enhancement.

We previously found the combination of cepharanthin and 5-fluorouracil or vinca alkaloids (vincristine, vinblastine, and vindesine) to have synergistic cytotoxicity against a human colon-cancer cell line (RPMI 4788) or a human uterine cervical cancer cell line (HeLa) in a modified microcytotoxicity-viable cell-staining assay [Ono et al., submitted for publication, 38].

Positive interactions between murine IFNs and chemotherapeutic agents have been reported. For instance, strong effects of IFN and cyclophosphamide (CY) were shown in AKR mice with spontaneous lymphoma [38], and the combination of IFN and 1,3-bis(2-chloro-ethyl)-1-ni-

trosourea was found to be curative in systemic murine leukemia [40]. However, Slater et al. [41] showed that IFN failed to enhance the response of L1210 leukemia to 6-mercaptopurine, Adriamycin, 1-β-D-arabinofuranosylcytosine, or CY, although some enhancement of the methotrexate response was seen.

The xenograft of human tumors in nude mice is an ideal system for evaluation of the direct and indirect antitumor action of IFN-cepharanthin combinations. In the present in vivo human tumor cell-nude mouse system, the antitumor effect of rIFN-γ or cepharanthin against RPMI 4788 transplanted into nude mice was shown as a significantly inhibitory effect against pulmonary metastasis in treated mice as compared with the control group. In the case of the combination of rIFN-γ with cepharanthin, the inhibitory effect was further strengthened in a synergistic manner (Table 5). However, the mechanism of the synergistic antitumor effect is not fully understood. Although it has been reported that cepharanthin increases the antitumor effect of anticancer agents on various tumor cells in vitro [16, 36, 37] and in vivo [16], no report exists on the combined effect of cepharanthin and IFNs.

Creasey et al. [42] reported that IFNs delayed cell transition from the G_0/G_1 phase to the S phase and prolonged the duration of the S phase. Ono [43] showed that when neoplastic cells were exposed to cepharanthin, they accumulated in the G_0/G_1 phase of the cell cycle. An arrest of the cell-cycle progression was also noted. These results suggest that the synergistic antitumor effects of rIFN-γ/rIFN-β and cepharanthin in combination should be more potent in terms of an action on cell-cycle progression than would be that of each agent alone. Furthermore, the action of IFNs is generally accepted to be based mainly on their direct antiproliferative activity against tumor cells [1] and on the augmentation of host-mediated immune responses [44]. IFN affects cell membranes and submembranes in many ways, such as by decreasing the cell-membrane fluidity [45, 46], increasing the quantity of microfilaments [46], decreasing cell motility [47], and increasing the proportion of membrane-bound proteins [48–52].

Verapamil, a calcium-channel blocker [53]; phenothiazine calmodulin inhibitors such as thioridazine [54]; and a synthetic isoprenoid [55] have all been found to enhance the cytotoxic effect of a conjugation of epidermal growth factor (EGF) with *Pseudomonas* exotoxin in HeLa cells. These agents are cationic and amphipathic and inhibit the degradation of EGF or low-density lipoprotein in lysosomes [56]. Cepharanthin is also cationic and amphipathic, and it potentiates the inhibitory effect on the degradation of EGF [20] and binds to phosphatidylserine in the membrane, resulting in the depression of membrane fluidity [14, 57]. The regulation of membrane fluidity by modulation of the cholesterol/phospholipid ratio affects the function of lymphocytes. The membrane-stabilizing action of cepharanthin is effective in potentiating the cytotoxic activity of anticancer drugs [16, 17, 19, 20]. Furthermore, cepharanthin exerts immunomodulatory effects by enhancing the cytotoxic effect of natural killer cells [21], antibody-dependent cellular cytotoxicity [58], and the activation of macrophages [22].

We conclude from the above discussion that interactions between IFNs and cepharanthin may have therapeutic potential and may be dependent on the dose and the duration of exposure to each agent. The present results also raise the possibility of the clinical use of IFN in combination with cepharanthin. Recently, rIFN-γ has also been used in clinical trials. The data reported herein suggest that administration of IFN and cepharanthin in combination might be very potent against various human tumor cells. The present study may give direction to future clinical trials of IFNs for the treatment of human malignancies with less toxicity via modulation with cepharanthin.

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