Jpn. J. Cancer Res. 84, 794–799, July 1993

Synergistic Antitumor Effects of Carboplatin and Interferons on Hepatic Metastases of Colon Carcinoma 26 and M5076 Reticulum Cell Sarcoma

Masaaki Iigo, Ishinori Shimizu and Katsuhiko Sagawa

Chemotherapy Division, National Cancer Center Research Institute, 1-1 Tsukiji 5-chome, Chuo-ku, Tokyo 104

The effects of combination therapy including various antitumor agents and interferon on mice bearing hepatic metastases of colon carcinoma 26 were determined. Combined treatment with interferon-αA/D and carboplatin (CBDCA) was associated with a considerably more pronounced antitumor effect than was treatment with either drug alone. Murine interferon-β and -γ each also potentiated the antitumor activity of CBDCA. Combination therapy with interferon-αA/D and CBDCA also resulted in marked inhibition of hepatic metastasis of M5076 reticulum cell sarcoma. However, interferon-β did not potentiate the antitumor activity of CBDCA against either subcutaneously implanted colon carcinoma 26 or pulmonary metastases of this tumor. Thus, in our model the combined administration of interferon and CBDCA was associated with a synergistic antitumor effect on hepatic metastases alone.

Key words: Hepatic metastasis — Colon carcinoma 26 — M5076 — Carboplatin — Interferon

Interferon-α and -β are currently available for clinical use. Both drugs are therapeutically efficacious in the treatment of certain types of leukemia and lymphoma, as well as of renal cell carcinoma. Their efficacy in the treatment of most other human solid tumors is, however, limited. Recently, it has been reported that interferons potentiate the antitumor effect of 5-fluorouracil[1] and that the metabolism of 5-fluorouracil may be modified by interferon.[2] Tumor cell function may be altered due to structural changes that increase the net negative charge on the cell surface[3] and changes in the density of the plasma membrane induced by interferon.[4] These changes may facilitate the penetration of some antitumor agents into tumor cells. In addition, interferon may induce various host-mediated immunomodulating effects, such as the augmentation of natural killer (NK) cell activity, which apparently play an important role in the prevention of metastasis.[5] These findings suggest that the combination of interferon with other commonly used antitumor agents may yield improved treatments for cancer.

In Japan, colorectal cancer has been increasing in frequency. In affected patients, hepatic metastasis may have a deleterious effect on survival and is associated with a low rate of response to systemic chemotherapy.[6] We therefore studied the therapeutic effect of antitumor agents administered in combination with interferon on hepatic metastasis in mice. In addition, we compared the antitumor activity of combination therapy against hepatic metastases of colon carcinoma 26 with that against pulmonary metastases and subcutaneously implanted colon carcinoma 26. Comparisons of the activities against these tumors may yield information useful for the selection of antitumor agents effective against tumors that metastasize to specific organs.[7]

MATERIALS AND METHODS

Chemicals We used cisplatin (Nippon Kayaku Co., Ltd., Tokyo), carboplatin (CBDCA, Bristol-Myers Squibb K.K., Tokyo), cyclophosphamide and vindesine (Shionogi & Co., Ltd., Osaka), doxorubicin, 5-fluorouracil and mitomycin-C (Kyowa Hakko Kogyo Co., Ltd., Tokyo) and pirarubicin (Meiji Seika Kaisha, Tokyo).

Animals Inbred, 5-week-old male BALB/c, CDF_1, C57BL/6 and BDF_1 mice weighing approximately 22 g each were obtained from Japan SLC (Hamamatsu). These were maintained under specific-pathogen-free conditions in our laboratory.

Tumor Colon carcinoma 26 was maintained *in vivo* by serial subcutaneous transplantation in BALB/c mice, and CDF_1 mice were used for experiments. For these experiments, tumors freshly excised from tumor-bearing mice were minced in Hanks' balanced salt solution (Life Technologies, Inc., Grand Island, NY) and strained through a 120 stainless-steel mesh. Viability was determined by trypan blue dye exclusion. Cells were diluted to the desired concentration. Six to eight mice were used for each dose tested. For subcutaneous implantation, colon carcinoma 26 cells were suspended and an aliquot containing 5×10^5 cells was injected into the right thigh of each mouse on day 0.

M5076 reticulum cell sarcoma (M5076) was maintained *in vivo* by serial intraperitoneal transplantation in C57BL/6 male mice, and BDF_1 mice were used for

experiments with this tumor. M5076 cells (3×10^6 cells/mouse) were injected into the tail vein on day 0. These mice were then randomized prior to allocation to the various experimental groups.

Experimental procedure for induction of hepatic metastases of colon carcinoma 26 Colon carcinoma 26 cells formed pulmonary metastases in mice following intravenous injection (2×10^4 viable tumor cells/mouse). However, multiple hepatic metastases of this tumor can be induced, as we have previously shown.[8] Briefly, mice were anesthetized with ether, a left subcostal incision (about 5 mm in length) was made, and the spleen was externalized. A 27-gauge needle (Terumo Japan, Tokyo), was used to puncture the splenic capsule, and 5×10^4 viable tumor cells suspended in 0.1 ml of Hanks' balanced salt solution were injected directly into the upper pole of the spleen. Gentle pressure was applied for a period of 10 s to prevent hemorrhage and tumor cell extravasation. The arteria and vena linealis were then clamped with a medium hemoclip (Edward Weck & Co., Inc., Research Triangle Park, NC), and the spleen was removed 20 s after the tumor cells had been implanted. The abdomen was sewn using surgical suture, and the skin was closed with disposable skin clip applicators (Avlox 12, Medi Plast, Sweden). The mice were allowed to recover and were then randomized prior to allocation to the different groups. Many metastases (more than 100), each approximately 0.3 mm in length, were found in the liver seven days after implantation. The lifespan of untreated CDF_1 mice bearing hepatic metastases was narrow in range; the mean survival time of the untreated mice was 18.5 ± 0.4 (SE) days ($n=111$). All dead animals had numerous metastases, and these were confined to the liver (liver weight of untreated controls at death $=5.60\pm0.13$ (SE) g, $n=111$; liver weight of normal mice $=0.99\pm0.01$ g, $n=60$).

Treatments Recombinant human interferon-αA/D, which was the product of a hybrid DNA of A and D clones, was a generous gift from Dr. Ishitsuka, Nippon Roche Research Center, Kamakura (specific activity: 2.04×10^7 IU/ml). Recombinant murine interferon-β (specific activity: 5.5×10^7 IU/mg protein) and interferon-γ (specific activity: 4.6×10^6 U/mg protein) were provided by Toray Industries, Inc., Kamakura. For experiments with colon carcinoma 26, these interferons were administered daily by intraperitoneal injection at doses of 1 to 2×10^5 IU/mouse on days 6 through 8 and 13 through 15. CBDCA was injected intravenously at 100 (maximum tolerated dose) and 50 mg/kg on days 7 and 14. The other antitumor agents were injected intravenously at their maximum tolerated doses on days 7 and 14. On the other hand, for experiments involving M5076, interferon-αA/D was administered by intraperitoneal injection at a dose of 2×10^5 IU/mouse on days 7, 11 and 15, CBDCA (50 mg/kg) was injected intravenously on days 7, 11 and 15. The animals were observed daily in order to determine survival time during a 100-day (colon carcinoma 26) or 120-day (M5076) follow-up period. The antitumor effect was determined by comparing the mean survival time of each group receiving treatment with that of the control group, and expressed as the increase in life-span (ILS). At the time of death of colon carcinoma 26-bearing mice, an autopsy was performed, and many hepatic or pulmonary metastases were observed in mice subjected to intrasplenic or intravenous injection of the tumor, respectively. The mice without tumor on day 100 were designated 'cured.' Autopsy was performed in order to check for the presence of tumor colonies in the liver or other organs in mice bearing M5076 at death (control and interferon-αA/D groups) or on day 120 (CBDCA and its combination groups).

Cell culture Colon carcinoma 26 cells to be cultured were prepared from tumor tissues. Cells were subcultured seven times and used for experiments. Cells were grown in 96-well tissue culture dishes (Falcon, Becton Dickinson Labware, Lincoln Park, NJ) at 37°C in a humidified atmosphere with 5% CO_2. The initial cell density was 5×10^3 cells per well. Each well contained 180 μl of Eagle's minimum essential medium supplemented with vitamins, sodium pyruvate, non-essential amino acids and L-glutamine with 10% fetal bovine serum (Gibco, Grand Island, NY). Twenty-four hours after the beginning of culture, graded concentrations of interferon-β (10 μl) and CBDCA (10 μl) were added. The cytotoxic effects of these drugs were assessed using the MTT test 24 h after their addition. Results were expressed as the relative percentage absorbance compared to that of the controls not receiving drugs. Absorbance was tested at 540 nm and measured using an Elisa Analyzer (ETY-96R, Toyo Sokki; Tokyo). Each experimental data point is the mean of quadruplicate determinations.

Statistical analysis The means of the various experimental groups were compared by using Student's t test.

RESULTS

The effects of antitumor agents, in combination with interferon-αA/D, -β and -γ, on survival of mice bearing established hepatic metastases were determined (Tables I–III). As shown in Table I, combined administration of CBDCA at 50 mg/kg and interferon-αA/D resulted in significantly greater prolongation of life than did administration of the single agents alone (Expt. I, $P=0.031$; Expt. II, $P=0.003$). When CBDCA at a dose of 100 mg/kg (maximum tolerated dose) was combined with interferon-αA/D, two out of six mice tested were cured;

Table I. Effect of Interferon-αA/D and Antitumor Agents in Combination or as Single Drugs on Hepatic Metastases of Colon Carcinoma 26-bearing Mice

Antitumor agent	Dose (mg/kg)	Interferon-αA/D	Survival[a] (days)	Increase in life-span (%)
Expt. I				
Control		−	19.7±0.9	
Adriamycin	12.5	−	42.6±2.2	116
Adriamycin	12.5	+	43.0±1.5	118
Pirarubicin	12.5	−	36.3±2.8	84
Pirarubicin	12.5	+	33.0±2.2	68
Cisplatin	5	−	32.5±3.2	65
Cisplatin	5	+	40.4±5.8(1/6)[b]	105
CBDCA	50	−	25.8±2.9	31
CBDCA	50	+	49.0±10.2*(2/6)[b]	149
Expt. II				
Control		−	16.3±0.6	
Interferon		+	23.3±2.6	43
Vindesine	2.5	−	17.3±2.3	6
Vindesine	2.5	+	26.5±3.7	63
Cyclophosphamide	100	−	34.5±2.7	112
Cyclophosphamide	100	+	32.0±1.6	96
CBDCA	50	−	20.5±1.6	26
CBDCA	50	+	33.5±3.1**(1/6)[b]	106
CBDCA	100	−	30.3±5.4	86
CBDCA	100	+	39.8±9.2(2/6)[b]	144

Interferon-αA/D (2×10^5 IU/mouse/day) was injected i.p. on days 6 through 8 and 13 through 15. Antitumor agents were injected i.v. on days 7 and 14.
a) Mean±SE (cured mice were excluded from calculations).
b) Number of cured mice per total number of treated mice, on day 100.
* $P<0.05$, ** $P<0.01$, compared to results obtained with the antitumor agent alone.

Table II. Effect of Murine Interferon-β and Antitumor Agents in Combination or as Single Drugs on Hepatic Metastases of Colon Carcinoma 26-bearing Mice

Antitumor agent	Dose (mg/kg)	Interferon-β	Survival[a] (days)	Increase in life-span (%)
Control		−	17.2±1.1	
Interferon-β		+	17.6±1.7	2
5-Fluorouracil	100	−	22.0±1.2	28
5-Fluorouracil	100	+	22.5±2.6	31
Mitomycin-C	5	−	26.5±1.8	54
Mitomycin-C	5	+	29.7±2.0	73
CBDCA	100	−	31.3±2.5	82
CBDCA	100	+	[39.7±8.1](3/6)[b]	[131]

Murine interferon-β (1×10^5 IU/mouse/day) was injected i.p. on days 6 through 8 and 13 through 15. Antitumor agents were injected i.v. on days 7 and 14.
a) Mean±SE (cured mice were excluded from calculation).
b) Number of cured mice per total number of treated mice, on day 100.

when either of the drugs was used alone, no mouse was cured. The antitumor activity of doxorubicin, pirarubicin or cyclophosphamide was not potentiated by interferon-αA/D. The antitumor activities of cisplatin and vindesine each tended to be potentiated by interferon-αA/D, though the differences between results obtained with antitumor agent alone and those in combination with interferon-αA/D were not statistically significant.

Interferon-αA/D (2×10^5 IU/mouse) had some antitumor activity against hepatic metastases of colon carcinoma 26, while interferon-β (2×10^5 IU/mouse) and interferon-γ (1×10^5 U/mouse) each had little effect (Tables II and III). Interferon-β also potentiated the antitumor activity of CBDCA (100 mg/kg); combination of these two agents resulted in the long-term survival of three out of six mice tested. On the other hand, the antitumor activity of 5-fluorouracil or mitomycin-C was not potentiated by interferon-β in this treatment regimen (Table II).

Interferon-γ also potentiated the antitumor activity of CBDCA, and combination therapy with these two agents resulted in significantly greater prolongation of life of hepatic metastasis-bearing mice than did treatment with CBDCA alone (Table III).

M5076 is known to form hepatic metastases when cells are injected into the tail vein.[9] The combination of interferon-αA/D and CBDCA also had a marked antitumor effect on hepatic metastases of M5076 (Table IV). On day 120, all mice given the combination therapy of CBDCA and interferon-αA/D had no tumor in any organ, while five of six mice tested with CBDCA alone

Table III. Effect of Murine Interferon-β and -γ on Antitumor Activity of Carboplatin against Hepatic Metastases of Colon Carcinoma 26

Drug	Survival (days) (mean±SE)	Increase in life-span (%)
Control	20.8±1.5	
Interferon-β (2×10^5 IU/mouse)	21.2±0.8	2
Interferon-γ (1×10^5 U/mouse)	21.0±1.8	1
Interferon-β+Interferon-γ	22.7±1.6	9
CBDCA (50 mg/kg)	24.2±1.6	16
CBDCA+Interferon-β	33.2±2.0**	60
CBDCA+Interferon-γ	35.3±4.7*(1/7)[a]	70

Murine interferon-β and -γ were injected i.p. daily on days 6 through 8 and 13 through 15. CBDCA (50 mg/kg) was injected i.v. on days 7 and 14.
a) Number of cured mice per total number of treated mice, on day 100. Cured mouse was excluded from calculation of survival.
* $P<0.05$, ** $P<0.01$, compared to result with CBDCA alone.

Table IV. Effect of Interferon-αA/D and CBDCA in Combination or as Single Drugs on M5076-bearing Mice

Drug	Survival[a] (days)	Increase in life-span (%)	Number of hepatic metastasis-bearing mice[b]/treated mice	Liver weight[a] (g)
Control	18.5±0.5	–	6/6	3.97±0.11
IFN-αA/D	20.5±1.1	11	6/6	3.91±0.14
CBDCA	>120	>549	5/6	3.93±1.39
CBDCA +IFN-αA/D	>120	>549	0/6	1.67±0.09

a) Mean±SE of six mice.
b) Number of hepatic metastasis-bearing mice and liver weights were determined at death in the case of Control and IFN-αA/D groups, or on day 120 in the case of CBDCA and CBDCA plus IFN-αA/D groups.

Table V. Effect of Interferon-αA/D and Antitumor Agents in Combination or as Single Drugs on Pulmonary Metastases of Colon Carcinoma 26-bearing Mice

Antitumor agent	Dose (mg/kg)	Interferon-αA/D	Survival[a] (days)	Increase in life-span (%)
Control		–	19.8±0.5	
Interferon-αA/D		+	21.8±1.3	10
Cisplatin	5	–	27.3±1.5	38
Cisplatin	5	+	28.4±2.5	43
CBDCA	100	–	29.7±3.6	50
CBDCA	100	+	30.0±3.1	52
CBDCA	50	–	23.0±1.0	16
CBDCA	50	+	24.5±1.3	24

Interferon-αA/D (2×10^5 IU/mouse/day) was injected i.p. on days 6 through 8 and 13 through 15. Antitumor agents were injected i.v. on days 7 and 14.
a) Mean±SE of survival of six mice.

had one or two large tumors in the liver. All mice treated with interferon-αA/D alone had numerous large hepatic metastases.

On the other hand, interferon did not potentiate the antitumor effect of CBDCA on subcutaneously implanted colon carcinoma 26. There were no significant differences between the antitumor activities of drugs given in combination or as single agents (mean survival days: control, 29.7±2.8 (SE) days; CBDCA, 40.5±3.3 days; interferon-β, 38.5±2.2 days; CBDCA+interferon-β, 42.3±4.7 days).

Metastasis following intrasplenic injection of colon carcinoma 26 was confined to the liver, while injection via the tail vein resulted in metastases confined to the lung. In experiments testing treatment of pulmonary metastases, no significant differences were found between the antitumor activity of drugs given in combination or as single agents (Table V).

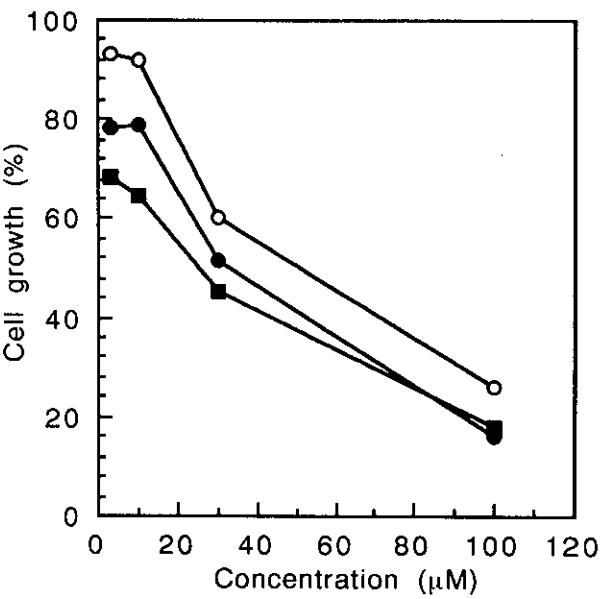

Fig. 1. Effect of interferon-β on CBDCA toxicity towards colon carcinoma 26 cells. Colon carcinoma 26 cells were incubated with varying concentrations of CBDCA in the absence of interferon-β (○), or in the presence of either 10^4 IU/ml (●) or 10^5 IU/ml of interferon-β (■). This procedure was repeated three times, and the results shown are those of a single typical experiment.

The growth-inhibitory effects of CBDCA and interferon-β against cultured colon carcinoma 26 are illustrated in Fig. 1. Interferon-β itself had no growth-inhibitory effect. The combination of interferon-β and CBDCA was more cytotoxic than was CBDCA alone. In particular, synergism of growth-inhibitory effect was observed for the combination of high concentrations of interferon-β and low concentrations of CBDCA.

DISCUSSION

Although interferons themselves have little direct inhibitory effect on tumor cells, the effect of certain antitumor agents is enhanced when they are used in combination with interferon, which may modify the matabolism of antitumor agents such as 5-fluorouracil.[2] Moreover, NK activity is augmented by interferon, and NK cells may have greater activity against tumor cells which have already been damaged by antitumor agents. The administration of interferon might therefore result in enhancement of the effect of antitumor agents currently in clinical use. Preclinical studies of the interactions between interferon and each of cyclophosphamide,[10] cisplatin[11-14] and vinblastin[15] have been reported.

Recently, cases of colorectal cancer have been increasing in Japan. This cancer frequently metastasizes to the liver. Therapy for hepatic metastasis is therefore required; systemic chemotherapy, however, has been found to be only minimally effective. In this study we attempted to determine which drugs might be potentiated by interferon as regards their effectiveness in the treatment of hepatic metastases. In our model of hepatic metastasis using colon carcinoma 26, the combination of an interferon with certain antitumor agents, in particular CBDCA, was associated with synergistic antitumor effects against hepatic metastases but not against subcutaneously implanted tumor or pulmonary metastases. The number of hepatic metastases as detected in histological examination of the liver five days after combination therapy with interferon and CBDCA was considerably less than that after treatment with either drug alone. Moreover, this combination had a marked antitumor effect on hepatic metastases of M5076. In an *in vitro* study using K562, the efficacy (in terms of cytotoxicity) of low concentrations of CBDCA was found to be enhanced by high concentrations of interferon.[16] On the other hand, although many reports have appeared on synergistic antitumor effects between cisplatin and interferon,[11-14] in our system no significant synergism was detected for these two agents. Interferon enhanced the efficacy against hepatic metastases of CBDCA alone, of various antitumor drugs tested. CBDCA has recently come into widespread clinical use. The mechanisms of action of CBDCA are probably similar to those of cisplatin.[17] However, because it possesses a stable dicarboxylate chelating structure, CBDCA has a slow rate of hydrolysis to the active form. It is believed that, following hydrolysis, CBDCA participates in the still slower formation of DNA interstrand cross-links and the slow disappearance of interstrand cross-links. CBDCA remains intact in the liver longer than does cisplatin.[18] Interferon-β is also concentrated in the liver following its administration.[19] Incorporation of CBDCA may be enhanced by interferon due to changes induced by the latter in the plasma membrane.[3,4] The potentiated antitumor activity of the combination of CBDCA and interferon in this system may be due to a direct effect on the tumor itself as well as effects on the host tissues. Although it is not clear why the antitumor activity of CBDCA alone, among the various antitumor agents tested, was potentiated by interferon, the effect is of interest, particularly since it was specific to hepatic metastases. Treatment of human patients with CBDCA and interferon in combination for hepatic metastasis may prove to be useful. Additional preclinical studies will be necessary to determine the optimal doses and treatment protocol for employment of CBDCA and interferon in combination therapy.

(Received February 24, 1993/Accepted March 29, 1993)

REFERENCES

1) Wadler, S. and Wiernik, P. H. Clinical update on the role of fluorouracil and recombinant interferon alfa-2a in the treatment of colorectal carcinoma. *Semin. Oncol.*, **17**, 16–21 (1990).
2) Elias, L. and Sandoval, J. M. Interferon effects upon fluorouracil metabolism by HL-60 cells. *Biochem. Biophys. Res. Commun.*, **163**, 867–874 (1989).
3) Knight, E., Jr. and Korant, B. D. A cell surface alteration in mouse L cells induced by interferon. *Biochem. Biophys. Res. Commun.*, **74**, 707–713 (1977).
4) Chang, E. H., Jay, F. T. and Friedman, R. M. Physical, morphological, and biochemical alterations in the membrane of AKR mouse cells after interferon treatment. *Proc. Natl. Acad. Sci. USA*, **75**, 1859–1863 (1978).
5) Brunda, M. J., Rosenbaum, D. and Stern, L. Inhibition of experimentally-induced murine metastases by recombinant alpha interferon: correlation between the modulatory effect of interferon treatment on natural killer cell activity and inhibition of metastases. *Int. J. Cancer*, **34**, 421–426 (1984).
6) Chu, D. Z. J., Hutchins, L. and Lang, N. P. Regional chemotherapy of liver metastases from colorectal carcinoma: hepatic artery or portal vein infusion? *Cancer Treat. Rev.*, **15**, 243–256 (1988).
7) Iigo, M., Nishikata, K., Nakajima, Y. and Araki, E. Effects of antitumor agents on subcutaneous implants and hepatic metastases of colon carcinoma 26 in mice. *Jpn. J. Cancer Res.*, **83**, 397–401 (1992).
8) Iigo, M., Nishikata, K., Nakajima, Y. and Hoshi, A. Effects of anthracycline derivatives on hepatic neoplastic

nodules of Lewis lung carcinoma and colon carcinoma 26. *Br. J. Cancer,* **63,** 363–366 (1991).
9) Hart, I. R. Seed and soil revisited. Mechanism of site-specific metastasis. *Cancer Metastasis Rev.,* **1,** 5–16 (1982).
10) Borden, E. C., Sidky, Y. A., Hatcher, J. F. and Bryan, G. T. Schedule-dependent variations in the response of murine P388 leukemia to cyclophosphamide in combination with interferon-α/β. *Cancer Res.,* **48,** 2329–2334 (1988).
11) Mowshowitz, S. L., Chin-Bow, S. T. and Smith, G. D. Interferon and cis-DDP: combination chemotherapy for P388 leukemia in CDF_1 mice. *J. Interferon Res.,* **2,** 587–591 (1982).
12) Inoue, M. and Tan, Y. H. Enhancement of actinomycin-D and *cis*-diamminedichloroplatinum (II)-induced killing of human fibroblasts by human β-interferon. *Cancer Res.,* **43,** 5484–5488 (1983).
13) Carmichael, J., Fergusson, R. J., Wolf, C. R., Balkwill, F. R. and Smyth, J. K. Augmentation of cytotoxicity of chemotherapy by human α-interferons in human non-small cell lung cancer xenografts. *Cancer Res.,* **46,** 4916–4920 (1986).
14) Sklarin, N. T., Chahinian, A. P., Feuer, E. J., Lahman, L. A., Szrajer, L. and Holland, J. F. Augmentation of activity of *cis*-diamminedichloroplatinum (II) and mitomycin C by interferon in human malignant mesothelioma xenografts in nude mice. *Cancer Res.,* **48,** 64–67 (1988).
15) Kuebler, J. P., Godette, G. A., Bock, D. J. and Epstein, R. B. Synergistic effects of vinblastine and recombinant interferon-beta on renal tumor cell lines. *J. Interferon Res.,* **10,** 281–291 (1990).
16) Hassan, H. T. and Maurer, H. R. Synergism between interferon-alpha and carboplatin in inhibiting K562 human myeloid leukaemia clonogenic cells: new potential therapeutic development. *Leuk. Res.,* **16,** 419–420 (1992).
17) Knox, R. J., Friedlos, F., Lydall, D. A. and Roberts, J. J. Mechanism of cytotoxicity of anticancer platinum drugs: evidence that *cis*-diamminedichloroplatinum(II) and *cis*-diammine (1,1-cyclobutanedicarboxylato)platinum (II) differ only in the kinetics of their interaction with DNA. *Cancer Res.,* **46,** 1972–1979 (1986).
18) Dible, S. E., Siddik, Z. H. and Harrap, K. R. The comparative tissues distribution of platinum and ^{14}C in mice receiving ^{14}C-labelled carboplatin. *Cancer Chemother. Pharmacol.,* **22,** 11–16 (1988).
19) Kasama, K., Satoh, Y., Usuki, K., Naruse, N. and Ida, N. Comparison of distribution between homologous and heterologous IFN in mice. *Proc. Jpn. Cancer Assoc., 43rd Annu. Meet.,* 318 (1984).