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In vivo activity on murine tumors of a novel antitumor compound, *N*-β-dimethylaminoethyl 9-carboxy-5-hydroxy-10-methoxybenzo[a]phenazine-6-carboxamide sodium salt (NC-190)

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Summary. A novel antitumor compound, N-β-dimethylaminoethyl 9-carboxy-5-hydroxy-10-methoxybenzo[a]phenazine-6-carboxamide sodium salt (NC-190) was evaluated for its antitumor activity in experimental murine tumor systems. In the initial studies with P388 leukemia (i.p.–i.p.), NC-190 led to an increase of >200% in life span (ILS), and 75% of the mice were alive on day 30, when the optimal dose (50 mg/kg, days 1–5) was given. Additionally, the compound had significant activities against i.p. inoculated mouse L1210 leukemia, B16 melanoma, M5076 reticulum cell sarcoma, sarcoma 180, mouse hepatoma MH134, and rat Yoshida sarcoma and Yoshida ascites hepatoma AH130. The optimal dose resulted in a >280% ILS with a 30-day survival of 50% in mice with L1210 leukemia (100 mg/kg, days 1–5), a 156% ILS in mice with B16 melanoma (50 mg/kg, days 1–5), a 98% ILS with a 90-day survival of 25% in mice with M5076 reticulum cell sarcoma (25 mg/kg, days 1, 5, 9, and 13), a >300% ILS with a 60-day survival of 50% in mice with sarcoma 180 (50 mg/kg, days 3–10), a 148% ILS with a 60-day survival of 25% in mice with MH134 (25 mg/kg, days 1–5), a 129% ILS with a 60-day survival of 12.5% in rats with Yoshida sarcoma (12.5 mg/kg, day 3–10), and a >161% ILS with a 60-day survival of 50% in rats with AH130 (6.3 mg/kg, days 3–10). In the experiments with s.c. inoculated tumors, NC-190 not only inhibited tumor growth, but also increased the life span of mice with Lewis lung carcinoma or B16 melanoma. The 60-day survivors accounted for 60% and 30% in mice with Lewis lung carcinoma and B16 melanoma, respectively. The compound significantly inhibited the spontaneous lung metastasis of Lewis lung carcinoma by more than 90% when eight daily i.v. injections were given. NC-190 was active by the i.p., s.c., and i.v. routes. Five consecutive daily i.p. doses (days 1–5) were more effective than a single dose (day 1), two doses (days 1 and 5), or three doses (days 1, 5, and 9). NC-190 warrants further study as a potential antineoplastic agent against human neoplasms, as it has a broad spectrum of antitumor activity and inhibits metastasis.

Introduction

With the availability of new drugs and the use of combinations of drugs, significant responses are being obtained in patients with certain types of cancer [1]. Despite advances in the treatment of cancer, tumors that are primarily refractory or that initially respond to chemotherapy but later recur remain one of the most frustrating yet challenging concerns for clinical oncologists. There is a great need for new and more effective antitumor agents.

N-β-Dimethylaminoethyl 9-carboxy-5-hydroxy-10-methoxybenzo[a]phenazine-6-carboxamide sodium salt (NC-190, Fig. 1) is a newly synthesized antitumor agent. The original active agent, *N*-β-dimethylaminoethyl 5-hydroxybenzo[a]-phenazine-6-carboxamide (NC-021), was discovered in random screening tests and led to the synthesis of a number of analogs. NC-190 was chosen for further development because it was the most active compound against P388 leukemia (manuscript in preparation). We evaluated NC-190 to determine its antitumor activity against a variety of transplantable tumors in vivo.

Materials and methods

Drugs. *N*-β-Dimethylaminoethyl 9-carboxy-5-hydroxy-10-methoxybenzo[a]phenazine-6-carboxamide sodium salt (NC-190; molecular weight 456.4) was synthesized in our laboratory (Fig. 1). A report on the synthesis and structure-activity relationships of this and related compounds is in preparation. For the study of its p.o. administration and the study with M5076 reticulum cell sarcoma, NC-190 was suspended in 5% or 0.5% gum arabic-0.9% NaCl solution. For other studies, it was dissolved in either 0.9% NaCl solution adjusted to pH 10.3 or 0.05 *M* carbonate buffer (pH 10.9). Mitomycin C (MMC) and adriamycin (ADM), formulated for clinical use, were obtained from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan). Cyclophosphamide (CPA), formulated for clinical use, was purchased from

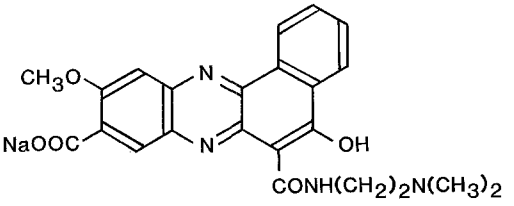

Fig. 1. Chemical structure of NC-190

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Abbreviations: ILS, increase in life span; *MST,* median survival time; *MMC,* mitomycin C, *ADM,* adriamycin; *CPA,* cyclophosphamide; *5-FU,* 5-fluorouracil

Shionogi Pharmaceutical Co., Ltd. (Osaka, Japan). 5-Fluorouracil (5-FU) was purchased from Sigma Chemical Co. (St. Louis, Mo.) MMC, ADM, 5-FU, and CPA were dissolved in 0.9% NaCl solution.

Animals and tumor cells. Female BALB/c × DBA/2 F_1 mice (hereafter termed $CD2F_1$; 6–8 weeks of age), female C57BL/6 × DBA/2 F_1 mice (hereafter termed $B6D2F_1$; 7–15 weeks of age), female C3H/HeN mice (7 weeks of age), female ICR mice (7 weeks of age), and female Donryu rats (6–7 weeks of age) were purchased from Charles River Japan (Kanagawa, Japan) and Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Japan). Food and drinking water were provided ad libitum. P388 leukemia, L1210 leukemia, M5076 reticulum cell sarcoma, and Lewis lung carcinoma were obtained from the Cancer Chemotherapy Center, Cancer Institute, Japanese Foundation for Cancer Research (Tokyo, Japan). Sarcoma 180 and mouse hepatoma MH134 were gifts from Prof. N. Tanaka, University of Tokyo, Japan. Yoshida sarcoma and Yoshida ascites hepatoma AH130 were gifts from The Sasaki Institute, Sasaki Foundation (Tokyo, Japan).

Evaluation of antitumor activity of ascitic tumors. Unless noted otherwise, tests were carried out according to protocols published by the National Cancer Institute (NCI, Bethesda, Md) [9]. P388 (1×10^6) cells and L1210 (1×10^5) cells were transplanted i.p. into $CD2F_1$ mice; B16 melanoma (0.5 ml 10% homogenate) cells and M5076 reticulum cell sarcoma (1×10^6) cells were inoculated i.p. into $B6D2F_1$ mice; sarcoma 180 (1×10^6) cells were inoculated i.p. into ICR mice; MH134 (1×10^6) cells were transplanted i.p. into C3H/HeN mice; Yoshida sarcoma (1×10^6) and AH130 (1×10^6) cells were inoculated i.p. into Donryu rats. In all, 8–16 animals were used in the control and 8 animals were used in drug-treated groups. Treatment (i.p., i.v., s.c., or p.o.) with NC-190 was initiated 1 or 3 days after tumor inoculation and was continued either daily for 5 or 8 days or every 4th day through day 13 (M5076). For the schedule dependency study, NC-190 was given i.p. to P388-inoculated mice on day 1 only; days 1 and 5; days 1, 5, and 9; or days 1–5. It was also injected i.p. on days 7–11 to evaluate its effect on the advanced stages of P388 leukemia.

Antitumor activity was assessed on the basis of the percentage of ILS. Median life spans were calculated from grouped median survival times (MST), and the percentage of ILS was calculated as

$$\text{ILS (\%)} = \frac{\text{MST of treated animals}}{\text{MST of control animals}} \times 100 - 100.$$

The criteria of effective activity in P388 leukemia, L1210 leukemia, B16 melanoma, and M5076 fibrosarcoma were the same as those used in NCI protocols [9]. There are no established criteria for sarcoma 180, MH134, Yoshida sarcoma, and AH130; therefore, the effects of the compound on these models were determined to be active when ILS reached > 100% according to Hoshino [5].

Evaluation of antitumor activity on solid tumors. B16 melanoma (0.5 ml 10% homogenate) cells and Lewis lung carcinoma (5×10^5) cells were implanted s.c. into $B6D2F_1$ mice, ten of which were used per group. NC-190 was given i.v. daily from day 3 to day 10. Antitumor activity was assessed according to the mean tumor volume, as derived from caliper measurements of the length and width of the tumor volume using the formula for a prolate ellipsoid,

$$\text{Tumor volume (mm}^3\text{)} = \frac{L \times W^2}{2},$$

in which L is the length in mm of the major axis and W is the length in mm of the minor axis. The T/C percentage of mean tumor volume was calculated:

$$\text{T/C (\%)} = \frac{\text{Mean tumor volume of treated animals}}{\text{Mean tumor volume of control animals}} \times 100.$$

Effect on spontaneous metastasis. Lewis lung carcinoma (5×10^5) cells in 0.025 ml Hanks' balanced salt solution (HBSS; Grand Island Biological Co., Grand Island, NY) was injected into the left hind footpad of $B6D2F_1$ mice on day 0. NC-190 was given i.v. on days 3–10 and CPA was given i.v. on days 3, 6, and 9. On day 12 after tumor inoculation, the left hind limb of each mouse (including the original tumor) was amputated. All mice were killed 21 days after the injection, and the lungs were removed and rinsed in 0.9% NaCl solution. The number of tumor colonies was determined by counting surface metastases under a dissecting microscope, since the majority of such metastases in mice are located near the lung surface [4]. Metastases were counted in a blind fashion by two observers.

Statistics. The results of solid tumor and spontaneous metastasis assays were analyzed using Dunnett's multiple comparison method [2] in cases of equal sample sizes. When the sample sizes were not equal, results were analyzed by Scheffe's multiple comparison method [7]. In Tables 4 and 5, values that differ from those of control groups at $P < 0.05$ are noted by a single asterisk and those that differ at $P < 0.01$, by a double asterisk.

Results

Effect of NC-190 against P388 leukemia

NC-190 given i.p. daily for 5 days, showed good activity against i.p. P388 leukemia in mice, consistently giving an ILS of > 100% and 30-day survivors over 10 separate dose-response studies. The results from one representative experiment are shown in Fig. 2. The results with ADM, MMC, and 5-FU were obtained from experiments done separately. The optimal dose of NC-190, 50 mg/kg, produced an ILS of > 200% and a large number (6 of 8 mice) of 30-day survivors. Even the dose of 0.1 mg/kg produced an ILS of 32%, and a 30-day survival was obtained with a dose of 6.25 mg/kg. ADM, MMC, and 5-FU, included as positive controls, led to ILS of 180% (2 of 8 animals survived 30 days), 157%, and 112%, respectively, at each optimal dose.

The dose response presented in Fig. 3 is a composite of four separate experiments in which the schedule dependency of NC-190 in the treatment of i.p. P388 leukemia was examined. When given i.p. the compound was active over a wide dose range on all schedules tested. An ILS of > 100% with 30-day survival of 25%–75% was obtained at doses of > 6.25 mg/kg on days 1–5 of treatment. Intermittent scheduling and a single injection resulted in a decreased efficacy but led to an ILS of > 100% with a 30-day survival of 13%–38%. The MTD of NC-190 was not

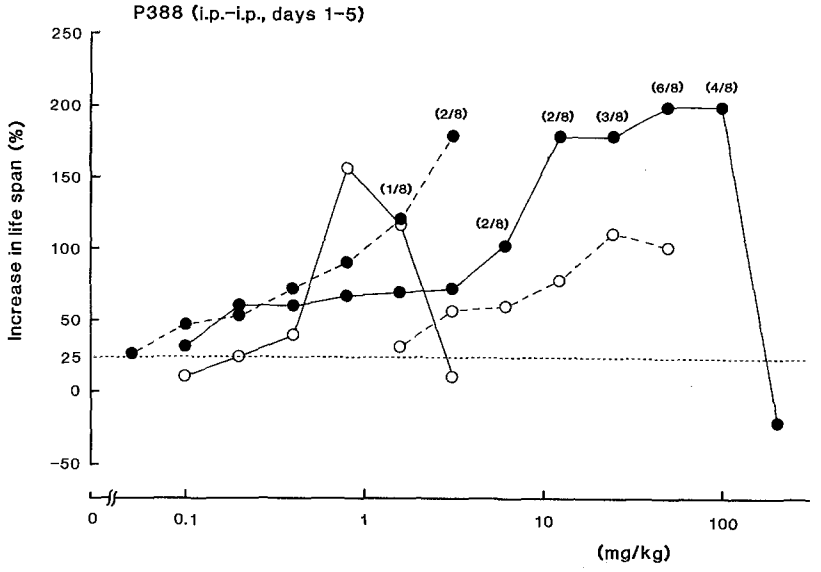

Fig. 2. Comparison of the effect of NC-190 and standard anticancer drugs against i.p. P388 leukemia. Tumor bearing mice were treated i.p. with NC-190 (●——●), MMC (○——○), ADM (●-----●), and 5-FU (○-----○) on days 1–5. Numbers in parentheses, 30-day survivors

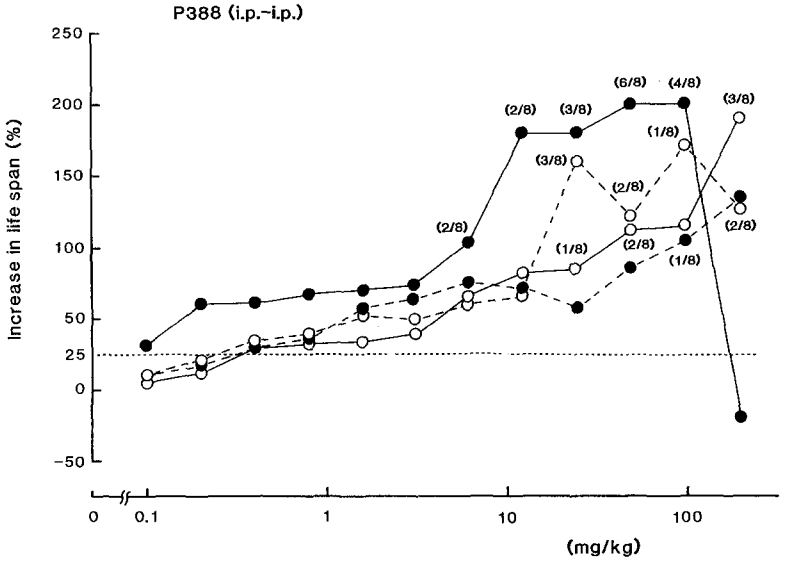

Fig. 3. Effect of administration schedule on the activity of NC-190 against i.p. P388 leukemia. Tumor bearing mice were treated i.p. with NC-190 on day 1 (○——○), days 1 and 5 (●-----●), days 1, 5, and 9 (○-----○), and days 1–5 (●——●). Numbers in parentheses, 30-day survivors

Table 1. Effect of the route of administration on the activity of NC-190 in mice bearing i.p. P388 leukemia

Dose[a] (mg/kg)	ILS (%) according to route of administration[b]			
	i.p.	i.v.	s.c.	p.o.
12.5	180 (2/8)[c]	50		
25	180 (3/8)	99	63	
50	>200 (6/8)	132	88	
100	>200 (4/8)		99	−2
200	−20			4
400				0

[a] NC-190 was given on days 1–5 following the i.p. implantation of 10^6 P388 leukemia cells

[b] Median survival times of control mice in four experiments ranged from 9.2 to 10.0 days

[c] 30-day survivors

achieved with day 1 or days 1 and 5 treatment; however, judging from the ILS and the dose range that produced 30-day survivors, the days 1–5 schedule is perhaps the most favorable for the administration of this compound.

The influence of the route of administration on the antitumor efficacy of NC-190 was then investigated (Table 1). With i.p. treatment, a distinct therapeutic advantage was noted. NC-190 was also effective when given i.v. and s.c., although there was a reduction in ILS values and there were no long-term survivors. In contrast, this compound was inactive when given p.o.

Additional studies using the i.p.-implanted P388 leukemia model were conducted to evaluate the effect of NC-190 against the advanced stage of this leukemia (Table 2). NC-190 and standard anticancer drugs were given i.p. daily from days 7 to 11. NC-190 was effective in increasing the survival of the host animals, although the ILS values were lower than those of the earlier stage treatment (days 1–5). CPA and 5-FU were also active, and CPA produced a 30-day survival of 25%. ADM was inactive in this model.

Effect of NC-190 on other ascites tumors

The antitumor activity of NC-190 was also evaluated in a variety of murine ascites tumor models. Included were L1210 leukemia, B16 melanoma, M5076 reticulum cell sar-

Table 2. Effect of NC-190 on the advanced stages of P388-bearing mice

Drug[a]	Dose (mg/kg)	Median survival (days)	ILS (%)
Control		9.1	0
NC-190	6.25	9.7	7
	12.5	11.0	21
	25	17.0	87
	50	17.8	96
	100	16.7	84
	200	15.6	71
ADM	1.6	9.7	7
	3.13	9.4	3
	6.25	10.0	10
CPA	50	21.0	131
	100	29.3	222 (2/8)[b]
	200	15.0	65
5-FU	12.5	12.8	41
	25	14.0	54
	50	16.1	77

[a] Drugs were given on days 7–11 following the i.p. implantation of 10^6 P388 leukemia cells
[b] 30-day survivors

Table 3. Response of i.p. implanted tumors to NC-190

Tumor[a]	Schedule	Final evaluation (day)	Optimal dose (mg/kg)	ILS[b] (%)
L1210	days 1–5	30	100	>280 (4/8)[c]
B16	days 1–5	90	50	156
M5076	days 1, 5, 9, 13	90	25	98 (2/8)
S180	days 3–10	60	50	>300 (4/8)
MH134	days 1–5	60	25	148 (2/8)
Yoshida	days 3–10	60	12.5	129 (1/8)
AH130	days 3–10	60	6.3	>161 (4/8)

[a] Tumor cells were implanted i.p. on day 0, as described in *Materials and methods*. NC-190 was injected i.p. according to the schedules shown
[b] Median survival times of control animals were 7.9 (L1210), 16.8 (B16), 27.8 (M5076), 15.0 (S180), 13.3 (MH134), 8.0 (Yoshida), and 23.0 (AH130) days, respectively
[c] Survivors on the final evaluation day

coma, sarcoma 180, MH134, Yoshida sarcoma, and AH130. From the schedule-dependency test on P388 leukemia, NC-190 was given daily i.p., except in M5076 reticulum cell sarcoma. As shown in Table 3, NC-190 was active in each of the above tumor models. L1210 leukemia, sarcoma 180, and AH130 were as responsive as P388 leukemia, and half of the treated animals in each group survived up to the final day of evaluation with optimal doses of NC-190. The compound was also active against B16 melanoma, M5076 reticulum cell sarcoma, MH134, and Yoshida sarcoma, increasing the life span by more than 98% following administration of the optimal doses.

Effect of NC-190 on solid tumors

NC-190 was also evaluated against s.c. inoculated B16 melanoma and Lewis lung carcinoma (Table 4). It showed

Table 4. Effect of NC-190 against s.c. implanted murine tumors

Tumor[a]	Dose[b] (mg/kg)	Tumor volume[c] T/C (%)	ILS[d] (%)	Survivors/ Total[e]	Body weight change (g)[f]
B16	Control	100	0	0/10	−0.1
	6.3	104	43	1/10	−0.1
	12.5	78	57	0/10	−0.2
	25	34*	74	3/10	0.2
Lewis	Control	100	0	0/10	0.4
	6.3	93	34	1/10	−0.1
	12.5	52	49	0/10	−0.4
	25	0*	>124	6/10	−0.4

[a] B16 melanoma cells (0.5 ml 10% homogenate) and Lewis lung carcinoma cells (5×10^5/mouse) were implanted s.c. into $BD2F_1$ mice
[b] NC-190 was injected i.v. on days 3–10
[c] On days 13 (B16) and 14 (Lewis)
[d] Median survival times of control mice were 34.3 (B16) and 26.8 (Lewis) days, respectively
[e] Survivors on day 60
[f] Mean body weights on days 10 or 11 minus mean body weight on day 3
* $P<0.01$

good activity against B16 melanoma after i.v. administration. A daily injection of 25 mg/kg on days 3–10 led to a significant reduction in tumor volume and increased the life span by 74%. No significant reduction in tumor volume was observed with 6.3 or 12.5 mg/kg, but survival was prolonged by 43% and 57%, respectively. ADM used as a positive control led to a significant reduction in tumor growth and increased the life span by 57% at a dose of 1.6 mg/kg (data not shown).

NC-190 showed better activity against Lewis lung carcinoma than against B16 melanoma. When 25 mg/kg was given, daily administration led to a significant reduction in tumor volume, and no tumor was palpable on day 14. Regrowth of the tumor in mice treated with 25 mg/kg NC-190 occurred as of day 20, but in 6 of 10 mice the tumor was not palpable even on day 60. Prolongation of the life span was observed at doses of 12.5 and 25 mg/kg, and the ILS values were 49% and >124%, respectively. ADM (0.4–1.6 mg/kg) was not effective in this model (data not shown).

Effect of NC-190 on the pulmonary metastasis of Lewis lung carcinoma

NC-190 was given i.v. from day 3 through day 10 after tumor inoculation. As shown in Table 5, 25 and 50 mg/kg NC-190 led to a significant inhibition of pulmonary metastasis. A dose of 12.5 mg/kg caused an inhibition of about 75% that was not statistically significant. CPA given on days 3, 6, and 9 significantly inhibited metastasis at doses of 50 and 100 mg/kg.

Discussion

Phenazine compounds were isolated from a culture filtrate of *Streptomyces griseoluteus* [6, 8]. Endo et al. [3] synthesized phenazine derivatives and noted their activity against mouse sarcoma 180. Among their compounds, 5-hydroxybenzo[a]phenazine was reported to inhibit the tumor

Table 5. Effect of NC-190 on the spantaneous metastasis of Lewis lung carcinoma[a]

Drug[b]	Dose (mg/kg)	Incidence of metastasis	Number of lung nodules	Weight of lung (mg/10 g body wt.)
Control		10/10	14.1 ± 2.6	104.8
NC-190	12.5	10/10	3.8 ± 0.9	75.8
	25	5/9	0.8 ± 0.4**	69.3**
	50	3/6	0.5 ± 0.3**	66.4**
CPA	25	9/9	13.2 ± 3.6	85.3
	50	9/10	1.8 ± 0.4**	77.6*
	100	7/10	0.9 ± 0.3**	83.8

[a] Lewis lung carcinoma cells (5×10^5/mouse) were implanted s.c. into the left foot pad on day 0. The left foot pad, including the primary tumor, was amputated on day 12, and the numbers of pulmonary tumor nodules were counted on day 21

[b] NC-190 was injected i.v. on days 3 – 10 and cyclophosphamide was given on days 3, 6, and 9

* $P<0.05$; ** $P<0.01$

growth of sarcoma 180 by 22%. However, the benzophenazine type of anticancer drug has not been developed for clinical use. We discovered a novel benzophenazine compound, NC-021, which showed marginal antitumor activity; among its derivatives, NC-190 was chosen as the most active compound. Because of its very low solubility at neutral pH, the compound was suspended in 5% or 0.5% gum arabic solution or dissolved in either 0.9% NaCl solution adjusted to pH 10.3 or 0.05 *M* carbonate buffer (pH 10.9). The alkaline solution was given to laboratory animals with no untoward effects, and there was no difference in the median survival or tumor growth rate between the 0.9% NaCl solution-treated group and the alkaline solution-treated group.

NC-190 showed significant activity against experimental tumors, including eight ascites tumors and five solid tumors in mice and rats. Under the conditions of treatment described in this report, NC-190 was more active than ADM against P388 leukemia and Lewis lung carcinoma. It was effective against i.p. inoculated P388 leukemia at doses from 0.1 to 100 mg/kg injected daily for 5 days, and its therapeutic range was about tenfold greater than that of ADM (Fig. 1). NC-190 showed a biphasic dose response: it was moderately active from 0.1 to 3.13 mg/kg; however, the dose was increased from 6.25 mg/kg, the activity rapidly elevated, reaching its maximum at 50 mg/kg. Furthermore, the compound was active against the advanced stage of P388 leukemia and inhibited the growth of Lewis lung carcinoma, a lesion unresponsive to ADM, at the doses given (Tables 2 and 4). These results suggest that the therapeutic dose range and antitumor spectrum in murine tumors are remarkable and worthy of further investigation.

It may be significant that NC-190 was active by i.v. injection against i.p. P388 leukemia (Table 1). Additionally, i.v. administration of the compound not only inhibited the growth of solid tumors, but also prolonged the life span of the mice (Table 4). The i.v. route of treatment may prove to be more relevant to the potential clinical use of this compound than other parenteral routes.

Although basic differences between transplanted tumor models and naturally occurring human tumors must be kept in mind, Lewis lung carcinoma has characteristics such as metastatic spread and relative resistance to most of the available cytotoxic drugs [9]. As mentioned above, 25 mg/kg NC-190 significantly inhibited tumor growth and prolonged the life span of Lewis lung carcinoma-inoculated mice. A moderate increase in life span was also observed at 6.3 and 12.5 mg/kg, doses which did not result in significant reductions in tumor volume (7% and 48%, respectively). These results suggest that NC-190 exerts antitumor activity by suppressing the formation of metastases as well as inhibiting growth of the primary tumor. Therefore, we examined the effects of NC-190 on the spontaneous pulmonary metastasis of Lewis lung carcinoma. We noted a significant inhibition of tumor metastasis of >90% at doses of 25 and 50 mg/kg, and a 73% inhibition was observed following 12.5 mg/kg treatment ($P>0.05$). The results on Lewis lung carcinoma (Tables 4 and 5) were obtained from two separate experiments, and the tumor-inoculation sites were varied in the tests. Thus, although it is difficult to compare the results directly, it is possible that NC-190 increased survival by not only suppressing the primary tumor but also inhibiting metastasis.

Preliminary evidence suggests that NC-190 is a DNA-reactive agent, as it inhibits DNA synthesis in HeLa S3 cells in vitro (manuscript in preparation).

As a new chemical agent with antineoplastic properties, NC-190 has significant and reproducible, broad-spectrum antitumor activities in murine tumor models.

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References

1. DeVita VT Jr (1982) Principles of chemotherapy. In: DeVita VT Jr, Hellmann S, Rosenberg SA (eds) Cancer, principles and practice of oncology. JB Lippinncott Co., Philadelphia, p 132
2. Dunnett CW (1964) New table for multiple comparisons with a control. Biometrics 20: 482
3. Endo H, Tada M, Katagiri K (1969) Studies on antitumor activity of phenazine derivatives against S180 in mice (VIII). Sci Rep Res Inst Tohoku Univ Ser C 16:18
4. Fidler IJ (1970) Metastasis: quantitative analysis of distribution and fate of tumor emboli labelled with ^{125}I-5-iodo-2′-deoxyuridine. J Natl Cancer Inst 45: 775
5. Hoshino A (1974) Screening systems and test models for cancer chemotherapy in Japan. Jpn J Cancer Chemother 1: 91
6. Osato T, Maeda K, Umezawa H (1954) The existence of griseoluteins A and B. J Antibiotics Ser A 7: 15
7. Scheffe H (1953) A method for judging all contrasts in the analysis of variance. Biometrika 40: 87
8. Umezawa H, Hayano S, Maeda K, Ogata Y, Okami Y (1950) A new antibiotic, griseolutein, produced by *Streptomyces*. Jpn Med J 3: 111
9. Venditti JM, Wesley RA, Plowman J (1984) Current NCI preclinical antitumor screening in vivo: Results of tumor panel screening, 1976–1982, and future directions. Adv Pharmacol Chemother 20: 1

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