IN VITRO ANTITUMOR ACTIVITY OF A NEW PLATINUM ANALOGUE, NK 121 AGAINST FRESH HUMAN TUMOR CELLS AND ESTABLISHED TUMOR CELL LINES BY SUCCINATE DEHYDROGENASE INHIBITION TEST

Hiroki Yamaue, Hiroshi Tanimura, Masaji Tani, Makoto Iwahashi, Takuya Tsunoda and Masaya Inoue

Department of Gastroenterological Surgery, Wakayama Medical College, 27-Shichibancho, Wakayama 640, Japan

(Received September 1, 1989 · Accepted April 19, 1990)

We analyzed the antitumor effect of a new platinum analogue, NK 121, against fresh human tumor cells and established tumor cell lines, and compared it with CDDP using the succinate dehydrogenase inhibition (SDI) chemosensitivity test. As the target cells, highly purified fresh tumor cells were obtained from three patients with malignant ascites due to gastric adenocarcinoma, two patients with pancreatic cancer, and two patients with ovarian cancer. The established KATO-III and C-1 cell lines were used for comparison. Tumor cells were cultured with 0.01—80 μg/ml of NK 121 or CDDP for 4 days, and following incubation the antitumor effect of NK 121 and CDDP was evaluated by the SDI test.

For fresh human tumor cells from patients treated with CDDP, the antitumor effect of NK 121 was superior to that of CDDP at concentrations below 5—10 μg/ml. In addision, for tumor cells from patients not treated with CDDP, the inhibition rate of NK 121 was similar to that of CDDP at concentrations less than 1—5 μg/ml. For KATO-III cells, the inhibition rate of CDDP was higher than that of NK 121 at concentrations above 20 μg/ml, but there was no difference in antitumor activity between the two agents under 10 μg/ml. For C-1 cells, there was also no difference between the two agents at concentrations less than 1 μg/ml.

Thus, NK 121 had a similar antitumor potency to CDDP at concentrations corresponding to the clinically achievable plasma levels which had been found in phase I trials.

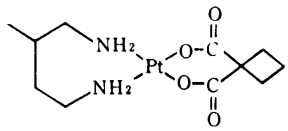
Key words : Platinum analogue, NK 121, Succinate dehydrogenase inhibition test, Cancer chemotherapy, Fresh human tumor cells

INTRODUCTION

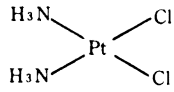
CDDP (cis-diamminedichloroplatinum II) is one of a group of platinum coordination complexes that was first described by Rosenberg et al.[1], and it has been widely used for cancer therapy because of its potential value as a chemotherapeutic agent[2~4]. The side effects of CDDP include renal toxicity[5], emetic reaction[6], ototoxicity[7] and myelosuppression[8], and the dose-limiting side effect is renal toxicity[5].

NK 121 is a novel platinum analogue (structural formula: cis-1, 1-cyclobutane dicarboxylate- (R) -2 -methyl-1, 4-butane diammine platinum II) developed for the purpose of reducing the side effects of CDDP (Fig. 1). In the past, it has generally been reported that the antitumor effects of CDDP analogues are inferior to that of CDDP itself[9].

Recently, the succinate dehydrogenase inhibition (SDI) test has been used to select effective anticancer agents for patients on an individual basis[10]. SDI



Cis-1, 1-cyclobutane dicarboxylate-(R)-2-methyl-1, 4-butane diamine platinum (NK 121)



Cis-diamminedichloroplatinum (CDDP)

Fig. 1. Chemical structures of NK 121 and CDDP

activity has been assayed in whole cell preparations without separation of tumor cells[11], and although the purity of tumor cells in resected specimens obviously varies widely. It is considered that the SDI test should be performed using highly purified tumor cells to improve its accuracy.

In this study, we compared the antitumor effects of NK 121 and CDDP by the SDI test, and NK 121 in clinical doses was shown to have *in vitro* antitumor activity against both highly purified human tumor cells and established tumor cell lines.

MATERIALS AND METHODS

1. Chemicals

CDDP and NK 121 were supplied by Nippon Kayaku Co., Ltd (Tokyo, Japan). NK 121 was provided as a lyophilized powder with a molecular weight of 439.37, which was dissolved in phosphate buffered saline. Both drugs were diluted in complete medium at a concentration of 0.01—80 μg/ml. The complete medium consisted of RPMI-1640 (Nissui Co., Tokyo, Japan) supplemented with 10 % heat-inactivated fetal calf serum (GIBCO, New York, USA), 2 mM L-glutamine, 100 units of penicillin/ml, and 100 μg of streptomycin/ml. The medium was adjusted to pH 7.2—7.4 using $NaHCO_3$.

2. Patient profiles

Case 1. A 57-year-old-male was admitted with advanced gastric cancer ($T_4N_3M_1$) in May 1988. Four months later, he developed malignant ascites, and was treated by intraperitoneal administration of CDDP on five occasions (total dose 500 mg). The clinical response to CDDP was evaluated as a partial response. He was re-admitted because of an increase in his ascites in February 1989.

Case 2. A 56-year-old male underwent total gastrectomy with lymph node dissection for gastric cancer ($T_3N_3M_0$) in March 1987. In October 1988, he was diagnosed as having multiple liver metastases, and was treated by the intravenous administration of CDDP on four occasions (total dose 300 mg). No clinical response to CDDP was recognized, and malignant ascites developed from February 1989.

Case 3. A 34-year-old female underwent 3 courses of intensive chemotherapy consisting of CDDP, adriamycin and cyclophosphamide (CAP) to treat peritoneal dissmination of ovarian cancer ($T_3N_1M_0$). In May 1989, she developed intestinal obstruction with ascites due to intra-abdominal recurrence, which was resistant to chemotherapy using CDDP.

Case 4. A 59-year-old female suffered from ovarian cancer and received 3 courses of CAP therapy. In June 1989, a metastasis to the hepatic hilum was detected, and this tumor was resected with partial hepatectomy ($T_3N_1M_0$).

Case 5. A 52-year-old female underwent partial gastrectomy with lymph node dissection ($T_3N_3M_1$) in February 1989. At operation, malignant ascites was noted to be present. As there had been no previous treatment with chemotherapeutic agents, ascitic fluid was collected during the operation.

Case 6. A 70-year-old male underwent pancreatoduodenectomy for pancreatic cancer ($T_2N_1M_0$) in February 1989. He had not previously been treated with chemotherapeutic agents. The resected tumor tissue was prepared for the following experiment.

Case 7. A 58-year-old male underwent distal pancreatectomy for pancreatic cancer ($T_2N_0M_0$) in September 1989. He also had not previously been treated by chemotherapeutic agents.

Thus, treatment with CDDP was performed in 4 of the 7 patients (cases 1—4), and no treatment had been performed in cases 5—7.

The ascites collected from cases 1, 2, 3 and 5, and the resected solid tumors from cases 4, 6 and 7 were used as the source of tumor cells.

Table 1. Patient profiles

Patient	Age/Sex	Diagnosis	Source of cells	CDDP treatment
1	57/M	gastric cancer	ascites	+
2	56/M	gastric cancer	ascites	+
3	34/F	ovarian cancer	ascites	+
4	59/F	ovarian cancer	primary tumor	+
5	52/F	gastric cancer	ascites	
6	70/M	pancreatic cancer	primary tumor	
7	58/M	pancreatic cancer	primary tumor	

A summary of the patients is shown in Table 1.

3. Purification of fresh human tumor cells

Malignant ascites was collected sterilely from four patients, immediately centrifuged at 400 g for 5 min, and then suspended in complete medium.

Freshly excised tumor tissues obtained from the other three patients were processed using enzymatic digestion, as described by Itoh et al.[12]. Briefly, tumor tissues were dissected into pieces smaller than 5 mm^3 which were immersed in complete medium containing collagenase (2 mg/ml, type V-S; Sigma), hyaluronidase (10 units/ml, type VI-S; Sigma), and DNase-I (0.4 mg/ml; Sigma). After a 40 min incubation at 37℃, the cells were harvested, washed three times, and suspended in complete medium.

The technique for purification of autologous tumor cells conformed to that of Uchida et al.[13]. Tumor cells obtained from ascites and solid tumor specimens were centrifuged on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradients at 400 g for 30 min. Mononuclear cells and tumor cells at the interface were collected, washed, and suspended at a concentration of 1×10^6/ml in complete medium. The cells were then layered on discontinuous gradients of 75 % and 100 % Ficoll-Hypaque. After centrifugation at 400 g for 30 min, a tumor cell-rich fraction was collected from the 75 % interface. The procedure was repeated if the initial separation was not successful, as judged by morphological examination. A tumor cell-enriched suspension was then layered onto discontinuons gradients containing 4 ml each of 25 %, 15 %, and 10 % Percoll (Pharmacia, Uppsala, Sweden) in complete medium. Centrifugation was performed at 25 g for 7 min, and cells were washed and suspended in complete medium at a concentration of 1×10^6/ml. The cells thus prepared were mainly tumor cells, with less than 10—15 % contamination by nonmalignant cells as judged by morphological examination. They were more than 90—95 % viable by the trypan blue dye exclusion test.

4. Established tumor cell lines

The KATO-III (human signet ring cell gastric carcinoma) and C-1 (human differentiated colon carcinoma) were used as established tumor cell lines. These cell lines were maintained in complete medium. On the day before the experiment, tumor cells were adjusted to a concentration of 5×10^5/ml in fresh complete medium and incubation was then continued at 37℃.

5. Method of SDI test

Chemosensitivity was assessed by the SDI test, using the tetrazolium salt MTT (3-(4, 5-dimethylthiazol)-2, 5-diphenyl tetrazolium bromide, Sigma No. M 2128) to measure the viability of tumor cells[14,15]. MTT was dissolved in phosphate buffered saline at 5 mg/ml and filtered for sterilization. Then, 100 μl of tumor cell suspension (5×10^5 cells/ml) was added to 25 μl of serial 2-fold dilutions of NK 121 or CDDP (0.01—80 μg/ml at final concentrations) in 96-well flat-bottomed microtiter plates (Corning No. 25860), and incubated at 37℃ in a humidified 5 % CO_2 atmosphere for 24—96 h. Each drug dilution was

assessed in triplicate. Three microtiter wells containing tumor cells suspended in 125 μl of complete medium (total tumor cell number was equivalent to that in test wells) were used as a control for cell viability, and three wells containing only complete medium were used as controls for nonspecific dye reduction. After incubation, 15 μl/well of MTT solution was added to all the wells, and plates were incubated for a further 4 h. Then acid-isopropanol (100 μl of 0.04 N HCl in isopropanol) was added to all the wells and the mixtures were pipetted thoroughly to dissolve the dark blue crystals.

The plates were then read on a microplate reader (Corona Electric, MTP-32) using a test wavelength of 570 nm and a reference wavelength of 630 nm. The control wells without tumor cells had an OD of less than 0.005. For the established tumor cell lines, the assay was performed three times, and the mean and standard deviation of the values obtained were determined. The inhibition rate was calculated as follows:

inhibition rate = (1 − OD drug treated/OD control) × 100

RESULTS

1. Purity of fresh human tumor cells

The purity of tumor cells immediately after enzymatic digestion alone or centrifugation alone was 22.2—51.9 % (mean ± SD, 37.2 ± 11.0 %), while after

Table 2. Purity of fresh human tumor cells

Patient	Tumor cell source	Purity of tumor cells (%)	
		Before purification	After purification
1	ascites	22.2	94.1
2	ascites	50.6	89.5
3	ascites	37.5	90.2
4	primary tumor	23.5	90.0
5	ascites	51.9	90.3
6	primary tumor	33.3	91.0
7	primary tumor	41.7	92.0
		37.2 ± 11.0	91.0 ± 1.5**

The tumor cells obtained from solid tumors or ascites were enriched to a purity of 91.0 ± 1.5% by purification.
The purity of tumor cells before purification (enzymatic digestion alone in solid tumors and centrifugation alone in ascites) was 37.2 ± 11.0% (**$p<0.01$).

Table 3. Optimal incubation time for the SDI test

Target cell : fresh human gastric carcinoma cell

Incubation time (h)	OD without drugs	Inhibition rate (%)	
		CDDP (80 μg/ml)	NK121 (80 μg/ml)
24	0.39	40.4	26.1
48	0.39	84.0	65.4
72	0.36	82.3	50.1
96	0.41	90.4	62.2

For fresh human tumor cells without drugs the OD_{570} was equivalent at each incubation time. The inhibition rate increased with incubation time.

processing on the Ficoll-Hypaque discontinuous gradients the purity was 70.5±6.8 %. Tumor cells were enriched to 91.0±1.5 % by use of the Percoll discontinuous gradients (Table 2).

2. Optimal incubation time for the SDI test

When fresh human tumor cells were incubated without drugs (case 1), the OD_{570} remained steady and there was no variation due to changes in incubation time. However, the inhibition rates for NK 121 and CDDP increased with longer incubation times (Table 3). Therefore, a 96-h incubation period was used for all subsequent studies.

3. Antitumor effects of NK 121 and CDDP against established tumor cell lines

For KATO III cells, there was no significant difference in OD_{570} between NK 121 and CDDP at under 10 μg/ml. However, 80 μg/ml of CDDP inhibited SDI activity completely, although NK 121 inhibited it only 56 % ($P<0.01$) (Fig. 2). For the C-1 line also, the inhibition of SDI activity by NK 121 was

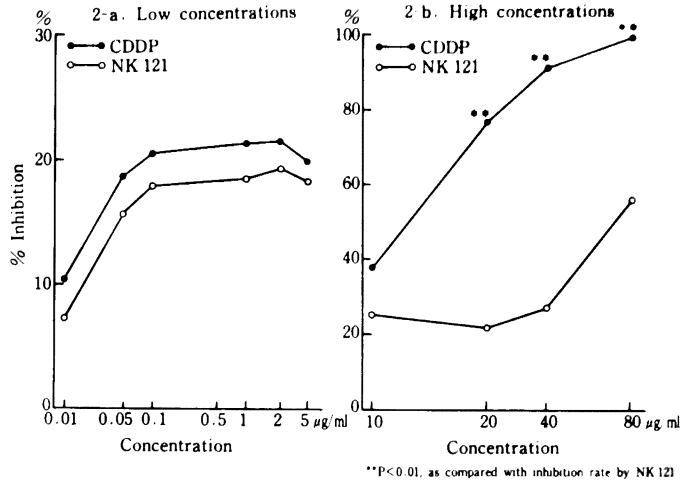


Fig. 2. Antitumor effects of NK 121 and CDDP against KATO-III cells

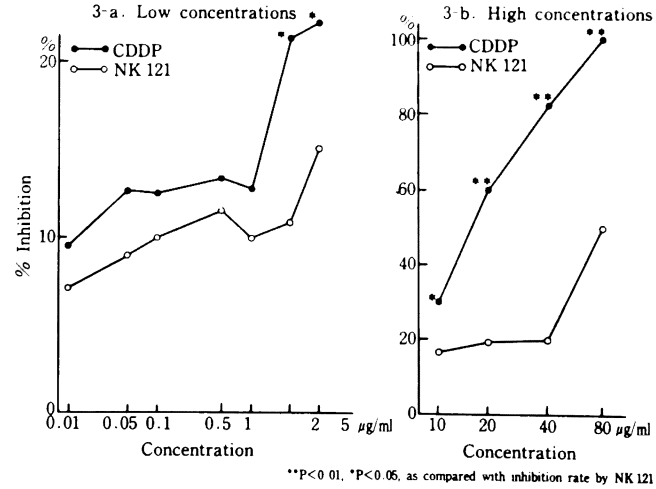
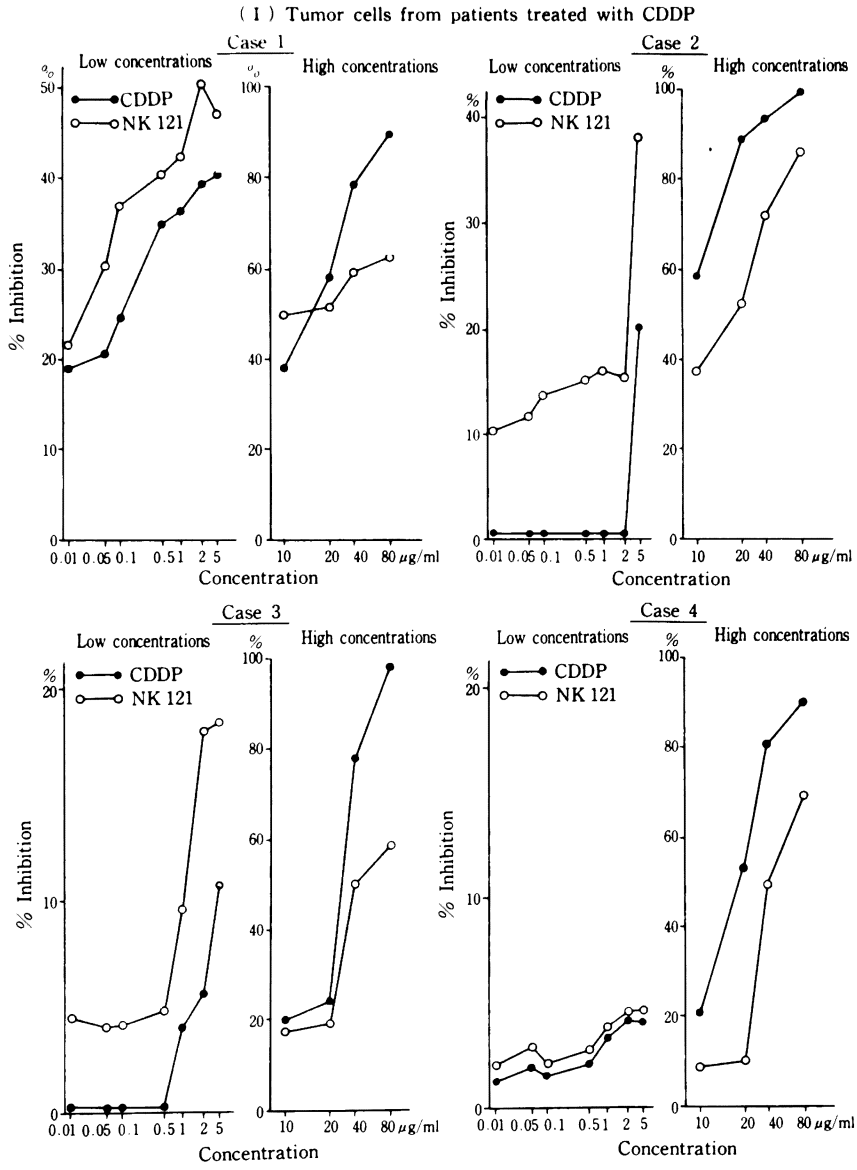


Fig. 3. Antitumor effects of NK 121 and CDDP against C-1 cells

equivalent to that obtained by CDDP under 1 μg/ml, whereas the viability of tumor cells was completely abrogated by a high concentration of CDDP in contrast to the effect of NK 121 ($P<0.01$ or 0.05) (Fig. 3).

4. Antitumor effects of NK 121 and CDDP against fresh human tumor cells

The purified tumor cells freshly isolated from the four patients treated with CDDP and the other three untreated patients were also evaluated for



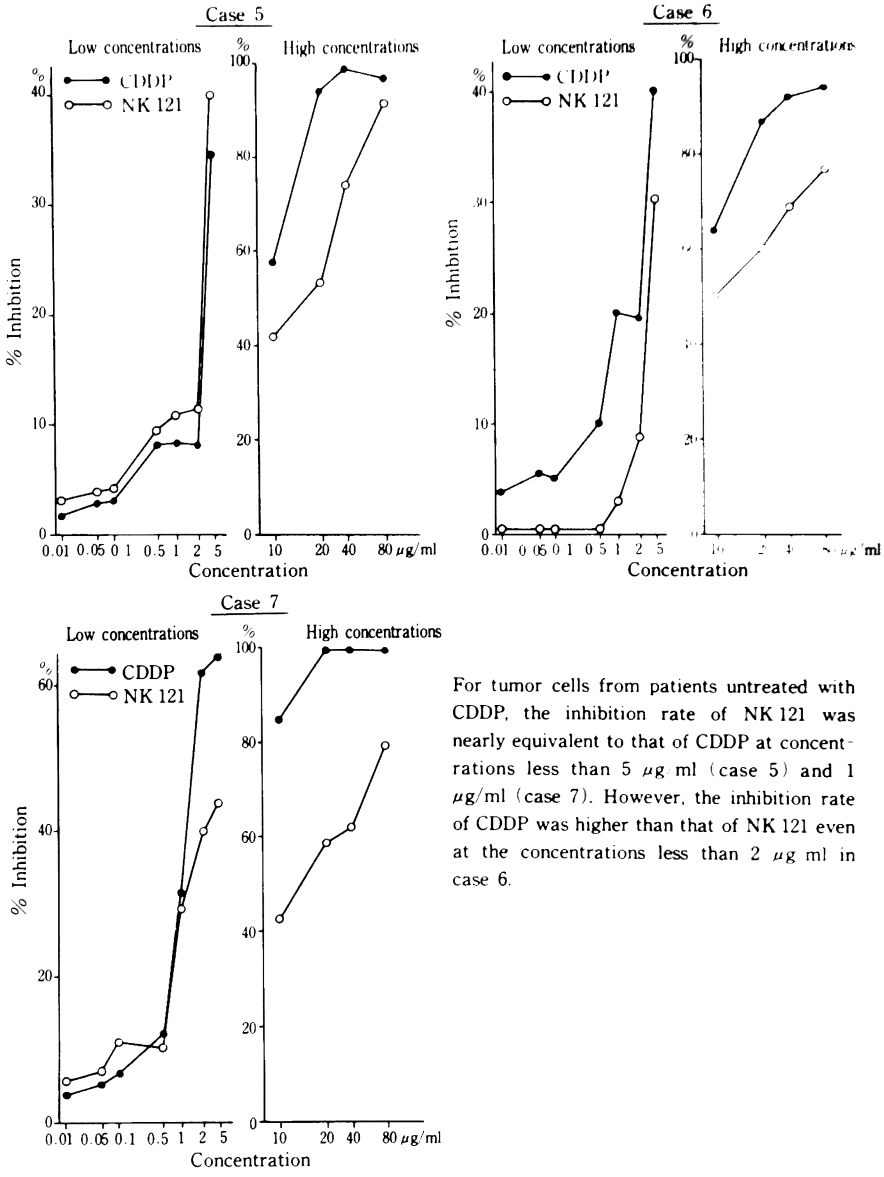
For tumor cells from patients treated with CDDP, the inhibition rate of NK 121 was higher than that of CDDP at concentrations less than 10 μg/ml (case 1) or 5 μg/ml (case 2 and 3). There was no difference between the inhibition rates of NK 121 and CDDP at concentrations less 5 μg/ml (case 4).

Fig. 4. Antitumor effects of NK 121 and CDDP against fresh human tumor cells

chemosensitivity. With tumor cells from case 1, the inhibition rate of CDDP was lower than that of NK 121 under 10 μg/ml, while it was higher at concentrations above 20 μg/ml. Similarly, in cases 2 and 3 the inhibition rate of NK 121 was better than that of CDDP under concentrations of 5 μg/ml. In addition, there was no difference between the inhibition rates of NK 121 and CDDP at concentrations less than 5 μg/ml in case 4 (Fig. 4).

In case 5, however, the inhibition rate of CDDP was nearly equivalent to that of NK 121 at concentrations under 5 μg/ml, while it was higher than that of NK 121 above 10 μg/ml. In case 6, the inhibition rate of CDDP was higher than that of NK

(II) Tumor cells from patients without treatment by CDDP



For tumor cells from patients untreated with CDDP, the inhibition rate of NK 121 was nearly equivalent to that of CDDP at concentrations less than 5 μg ml (case 5) and 1 μg/ml (case 7). However, the inhibition rate of CDDP was higher than that of NK 121 even at the concentrations less than 2 μg ml in case 6.

Fig. 5. Antitumor effects of NK 121 and CDDP against fresh human tumor cells

121 even at concentrations less than 2 μg/ml. In case 7, there was no difference between the antitumor effect obtained by NK 121 and CDDP at a concentration of less than 1 μg/ml (Fig. 5).

DISCUSSION

Recently, CDDP has become one of the major agents used in cancer chemotherapy. At present, renal toxicity remains the dose-limiting side effect, though other side effects have been reduced by the use of diuretics, anti-emetic agents, and fosfomycin[16~18].

Because of its toxicity, several analogues of CDDP have been developed in an effort to ameliorate its side effects while retaining the antitumor activity of the parent compound.

The side effects of these new analogues of CDDP are less than those of the parent compound, but Russell et al. have demonstrated that at equivalent concentrations carboplatin and iproplatin were both about 10 times less toxic than CDDP for tumor cells[19]. Other investigators have also reported that amino-ethylpyrrolidine-platinum complexes had a 2- to 3-fold weaker toxic effect than CDDP[20].

A phase I study of NK 121 was performed in 38 cancer patients, and no significant nephrotoxicity was observed. Nausea and vomiting were noted in most of the patients who received ≧320 mg/m², but only three patients required anti-emetic agents[21].

It was found that 80 % of NK 121 was not bound to protein until 8 h after a 3-h drip infusion. The C_{max} at a dose of 80—120 mg/m² was 7—13 μg/ml, and at 200—280 mg/m² it was 20—26 μg/ml. Plasma levels of NK 121 were maintained at 1 μg/ml at 4 h or 8 h after the administration of 80—120 mg/m² or 200—280 mg/m² of NK 121, respectively (unpublished data).

The present study showed that NK 121 had an equally potent antitumor effect against highly purified human tumor cells as did CDDP at concentrations less than 5 or 10 μg/ml in cases 1—5. These levels are similar to the plasma levels maintained for 4—8 h by a clinical dose of CDDP or NK 121[21,22]. Moreover, when the cut-off concentrations were set at 20 μg/ml for CDDP and 80 μg/ml for NK 121 on the basis of the drug pharmacokinetics, the inhibition rates for both drugs were over 50 %. Thus, it was suggested that the antitumor effects of CDDP and NK 121 were equivalent at clinically achievable concentrations.

In previous SDI tests used to determine chemosensitivity, tumor cells were not purified and whole cell preparations including lymphocytes and mesothelial cells were used[10,11,23]. We found that the purity of tumor cells was from 22 % to 52 % immediately after enzymatic digestion of tumor tissues, and was increased to 91 % by the purification technique described in this paper. To clarify the antitumor effect of a chemotherapeutic agent against fresh human tumor cells, it is important to exclude nonmalignant cells as far as possible when performing the SDI test.

In the established tumor cell lines which contained no nonmalignant cells (KATO-III and C-1), it was found that NK 121 inhibited the viability of tumor cells as potently as CDDP at concentrations less than 1—5 μg/ml.

Other investigators have demonstrated that the therapeutic efficacy of NK 121 was equivalent to that of CDDP in certain human tumor cell lines transplanted into nude mice[24]. In contrast, using a colony assay, Yonei et al. reported that the antitumor effect of NK 121 was weaker than that of CDDP against established tumor cell lines and fresh human tumor cells[25].

Moreover, Lokich et al. demonstrated that the patterns and frequency of toxicity were greatly diminished, and the excellent antitumor effects were observed by continuous administration of CDDP[26]. Thus, it is suggested that NK 121 may offer the therapeutic efficacy by continuous venous infusion, preserving a relatively low plasma concentration.

It is of interest that the inhibition rate of NK 121 was higher than that of CDDP at concentrations less than 5—10 μg/ml in fresh tumor cells obtained from patients who had been treated with CDDP. Also, it was nearly equal to that of CDDP in tumor cells from case 5 and 7 not previously treated with CDDP (at concentrations less than 1—5 μg/ml). These results suggest that there is no cross-reactivity between NK 121 and CDDP, and we are currently investigating whether NK 121 has antitumor activity against CDDP-resistant tumor cell lines.

References

1) Rosenberg B, Van Camp L, Krigas T: Inhibition of cell division in *Escherichia coli* by electolysis products from a platinum electrode. Nature, 205: 698～699, 1965
2) Katz M E, Schwartz P E, Kapp D S, Luikart S: Epithelial carcinoma of the ovary: current strategies. Ann. Int. Med., 95: 98～111, 1981
3) Kelson D P, Bains M, Hilaris B, Chapman R, Mccormack P, Alexande J, Hopfan S, Martini N: Combination chemotherapy of esophageal carcinoma using cisplatin, vindesine, and bleomycin. Cancer, 49: 1174～1177, 1982
4) Ozols R F, Behrens B C, Ostchega Y: High dose cisplatin and high dose carboplatin in refratory ovarian cancer. Cancer Treat. Rev., 12: 59～65, 1985
5) Blachley J D, Hill J B: Renal and electrolyte disturbance ssociated with cisplatin. Ann. Int. Med., 95: 628～632, 1981
6) Kris M G, Gralla R J, Tyson L B, Clark R A, Kelsen D P, Reilly L K, Groshen S, Bosl G J, Kalman L A: Improved control of cisplatin-induced emesis with high dose metoclopromide and with combinations of metoclopramide, dexamethasone, and diphenhydramine. Cancer, 55: 527～534, 1985
7) Helson L, Okonkwo E, Anton L: Cisplatin ototoxicity. Clin. Toxicol., 13: 469～478, 1978
8) Von Hoff D D, Schilsky R, Reichert C M, Reddick R L, Rozencweig M, Young R C, Mugga F M: Toxic effects of cis-dichlorodiammineplatinum (II) in man. Cancer Treat. Rep., 63: 1527～1531, 1979
9) Harrap K R, Jones M, Wilkinson C R, Clink H M, Sparrow S, Mitchley B C V, Clarke S, Veasey A: Antitumor, toxic and biochemical properties of cisplatin and eight other platinum complexes. In: Prestayko A W, Crooke S T, Carter S K, Alder N A (eds.), Cisplatin. Current status and new developments. pp. 193～212. New York, Academic Press, Inc., 1980
10) Carmichael J, Degraff W G, Gazder A F, Minna J D, Mitchell J B: Evaluation of a tetrazolium-based semiautomated colorimetric assay: Assessment of chemosensitivity testing. Cancer Res., 47: 936～942, 1987
11) Maehara Y, Anai H, Kusumoto H, Kusumoto T, Sugimachi K: Colorectal carcinoma *in vitro* is more sensitive to 1-hexylcarbamoyl-5-fluorouracil compared with six other antitumor drugs: carboquone, adriamycin, mitomycin C, aclacinomycin A, cisplatin, 5-fluorouracil. Dis. Col. Rec., 31: 62～67, 1988
12) Itoh K, Tilden A B, Balch C M: Interleukin 2 activation of cytotoxic T-lymphocytes infiltrating into human metastatic melanomas. Cancer Res., 46: 3011～3017, 1986
13) Uchida A, Micksche M: Lysis of fresh human tumor cells by autologous large granular lymphocytes from peripheral blood and pleural effusions. Int. J. Cancer, 32: 37～44, 1983
14) Mosmann T: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods, 65: 55～63, 1983
15) Green L M, Reade J L, Ware C F: Rapid colorimetric assay for cell viability: application to the quantitation of cytotoxic and growth inhibitory lymphokines. J. Immunol. Methods, 70: 257～268, 1984
16) Pera M F, Zook B C, Harder H C: Effects of mannitol or furosemide diuresis on the nephrotoxicity and physiological disposition of cis-dichlorodiamineplatinum (II). Cancer Res., 39: 1269～1278, 1979
17) Litterst C L: Alterations in the toxicity of cis-dichlorodiammineplatinum-II and in tissue localization of platinum as a function of NaCl concentration in the vehicle of administration. Toxicol. Appl. Pharmacol., 61: 99～108, 1981
18) Schweitzer V G, Dolan D F, Abrams G E, Davidson T, Snyder R: Amelioration of cisplatin-induced ototoxicity by fosfomycin. Laryngoscope, 96: 948～958, 1986
19) Russell J, Adam J, Wheldon T E, Kaye S B: The relative effectiveness of analogues of cisplatin in the experimental chemotherapy of human non-small-cell lung cancer and neuroblastoma grown as multicellular spheroids. Cancer Chemother. Pharmacol., 23: 111～114, 1989
20) Khokhar A R, Brown D B, McCormack J J, Hacker M P: Synthesis and antitumor activity of a series of (amino-ethylpyrrolidine) platinum complexes. Cancer Chemother. Pharmacol., 23: 15～18, 1989
21) Fukuoka M, Niitani H, Hasegawa K, Majima H, Hino M, Furue H, Tsukagoshi S, Fujita H, Ohta K, Furuse K, Kimura I, Katoh T: Phase I study of new platinum compound, NK 121. Abst. in A. S. C. O., p, 62, 1989
22) van Hennik M B, van der Vijgh W J F, Klein I, Elferink F, Vermonken J B, Winograd B, Pinedo H M: Comparative pharmacokinetics of cisplatin and three analogues in mice and humans. Cancer Res., 47: 6297～6301, 1987
23) Kanematus T, Maehara Y, Kusumoto T, Sugimachi K: Sensitivity to six antitumor drugs differs between primary and metastatic liver cancers. Eur. J. Clin. Oncol., 24: 1511～1513, 1988

24) Sawada M, Ozaki M, Taniguchi H, Tateishi R, Mori Y, Mino K: Effect of a newly developed platinum compound cis-1, 1-cyclobutane dicarboxylato-(2 R)-2-methy-1, 4-butane diammine platinum (II), on human ovarian tumors transplanted into nude mice. Jpn. J. Cancer Chemother., 15: 637～641, 1988 (in Japanese)
25) Yonei T, Ohnoshi T, Hirai S, Ueoka H, Yamashita H, Kozuka A, Moritaka T, Uji H, Kiura K, Mima Y, Kimura I: Comparison of antitumor activity of newly developed platinum analogues in lung cancer using colony assay. Jpn. J. Cancer Chemother., 16: 427～430, 1989 (in Japanese)
26) Lokich J J: Phase I study of cis-diamminedichloro-platinum (I) administered as a constant 5-day infusion. Cancer Treat. Rep., 64: 905～908, 1980

新しいCDDP誘導体NK 121のSuccinate dehydrogenase inhibition法を用いたヒト新鮮分離腫瘍細胞および培養癌細胞に対する *in vitro* 抗腫瘍効果の検討

山上 裕機・谷村 弘・谷 眞至
岩橋 誠・角田 卓也・井上 正也
和歌山県立医科大学消化器外科*

新しいCDDP誘導体であるNK 121の新鮮ヒト癌細胞および培養癌細胞に対する抗腫瘍効果をCDDPを対照としてSDI法による抗癌剤感受性試験を用いて解析した。胃癌による癌性腹水3例，卵巣癌2例および膵癌2例からFicoll-Hypaque 2段階・Percoll 3段階不連続密度勾配法により純度90％以上で単離した新鮮ヒト腫瘍細胞と，胃印環細胞癌KATO-III，大腸癌株化細胞C-1について，0.01～80 μg/mlのCDDPまたはNK 121を加えて4日間のSDI法を施行した結果，CDDPの治療歴のない3例中2例では，1～5 μg/ml以下の低濃度においてNK 121とCDDPの抑制率に差を認めなかったが，CDDPによる治療歴のある4例中3例で，5～10 μg/ml以下の低濃度においてNK 121の抑制率はCDDPのそれを上回った。これに対し，培養細胞では，KATO-IIIに対しては20 μg/ml以上の濃度でCDDPの抑制率はNK 121より高値であったが，10 μg/ml以下で両薬剤に差を認めず，C-1に対しても1 μg/ml以下の濃度で両薬剤に差を認めなかった。

したがって，NK 121の第1相試験で得られた薬剤の低い血中濃度の持続状態においては，NK 121はCDDPと同等の抗腫瘍活性を発現し，臨床的な有用性が特に発揮されると思われた。

*和歌山市七番丁 27