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Abstract

Antitumor activities of five platinum analogs, including cisplatin, carboplatin, 254-S, DWA2114R, and NK121, were compared using five human lung cancer cell lines and 19 tumor specimens obtained from lung cancer patients. The antitumor activity was evaluated by determining the ratio of the maximum tolerated dose of each drug to the 70% tumor growth inhibitory concentration in a colony assay. Cisplatin was the most potent agent, followed by 254-S and carboplatin. DWA2114R and NK121 were less potent than cisplatin and 254-S. Cross-resistance to adriamycin was also investigated using an adriamycin-resistant small cell lung cancer subline, SBC -3/ADM30. SBC-3/ADM30 was 1.7- to 4.0-fold more resistant to cisplatin, carboplatin, NK121, and DWA2114R, than was the parent line, SBC-3, and the subline was 2.0-fold more sensitive to 254-S. Using SBC-3, in vitro combination effects of etoposide and cisplatin, carboplatin, or 254-S were evaluated by the median-effect principle. Synergism was noted when cisplatin and etoposide were combined at a fixed molar ratio of 1:1. Combination of carboplatin and etoposide showed an additive effect. The combination of 254-S and etoposide was antagonistic at low concentrations, but was markedly synergistic at higher concentrations. These data suggested the efficacy of 254-S in the treatment of lung cancer.

KEYWORDS: platinum analogs, antitumor activity, lung cancer, colony assay, combination effect

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Antitumor Activity of Platinum Analogs against Human Lung Cancer Cell Lines and Tumor Specimens

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Antitumor activities of five platinum analogs, including cisplatin, carboplatin, 254-S, DWA2114R, and NK121, were compared using five human lung cancer cell lines and 19 tumor specimens obtained from lung cancer patients. The antitumor activity was evaluated by determining the ratio of the maximum tolerated dose of each drug to the 70% tumor growth inhibitory concentration in a colony assay. Cisplatin was the most potent agent, followed by 254-S and carboplatin. DWA2114R and NK121 were less potent than cisplatin and 254-S. Cross-resistance to adriamycin was also investigated using an adriamycin-resistant small cell lung cancer subline, SBC-3/ADM$_{30}$. SBC-3/ADM$_{30}$ was 1.7- to 4.0-fold more resistant to cisplatin, carboplatin, NK121, and DWA2114R, than was the parent line, SBC-3, and the subline was 2.0-fold more sensitive to 254-S. Using SBC-3, *in vitro* combination effects of etoposide and cisplatin, carboplatin, or 254-S were evaluated by the median-effect principle. Synergism was noted when cisplatin and etoposide were combined at a fixed molar ratio of 1:1. Combination of carboplatin and etoposide showed an additive effect. The combination of 254-S and etoposide was antagonistic at low concentrations, but was markedly synergistic at higher concentrations. These data suggested the efficacy of 254-S in the treatment of lung cancer.

Key words : platinum analogs, antitumor activity, lung cancer, colony assay, combination effect

In 1969, Rosenberg *et al.* reported the excellent antitumor activity of cisplatin (1) and the compound was introduced into clinical trials by the National Cancer Institute in 1972. It is now covincingly clear that cisplatin plays a significant role in the treatment of a wide variety of malignancies, such as genito-urinary tumors, head and neck tumors, and lung cancer. Cisplatin combined with etoposide has been shown to have definite activity and is now used as front-line chemotherapy for small cell lung cancer (SCLC) (2-4). However, the toxicities of cisplatin, particularly renal and gastrointestinal toxicity, often limit the dosage. Numerous platinum analogs have been synthesized in search for more potent and less toxic compounds (5-7). One of these, carboplatin is recognized to be active against SCLC. Three new platinum analogs, 254-S (8-11), DWA2114R (12), and NK121 (13-14), have recently been developed in Japan and are now undergoing clinical trials. We compared the antitumor activity of these platinum analogs with that of cisplatin and carboplatin by an *in vitro* colony assay using human lung cancer cells. We also investigated the cross-resistance pattern of these drugs to adriamycin using an adriamycin-resistant SCLC subline. Further, we evaluated the effects of cisplatin, carboplatin, or 254-S, when combined with etoposide using a SCLC cell line. The combination effects were quantitatively analyzed according

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to the median-effect principle in order to determine which drug combination was the most synergistic.

Materials and Methods

Chemical agents. Chemical structures of the five platinum analogs studied are shown in Fig. 1. Cisplatin, carboplatin, and etoposide were obtained from Bristol-Myers Research Institute Ltd. (Tokyo, Japan), 254-S was from Shionogi Pharmaceutical Co., Ltd. (Osaka, Japan), NK121 was from Nippon Kayaku Co., Ltd. (Tokyo, Japan), and DWA2114R came from Chugai Co., Ltd. (Tokyo, Japan). The clinical formulation of each agent was used in this study. All the drugs were dissolved and diluted to the required concentrations with 3% mannitol immediately before use.

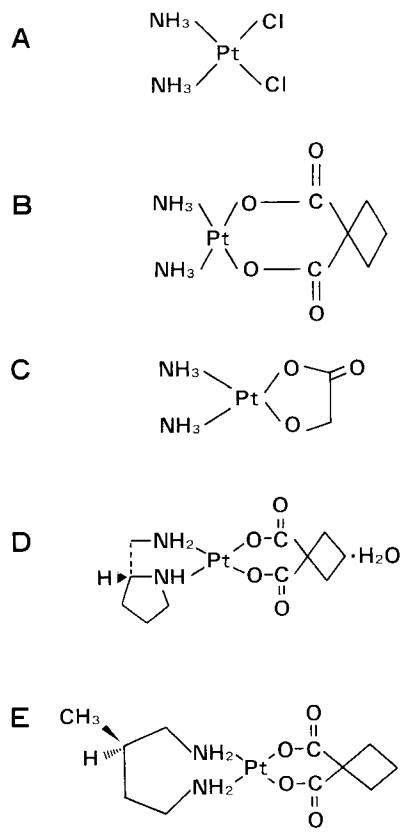

Fig. 1 The chemical structures of platinum analogs used in the study.
A: Cisplatin (CDDP) *cis*-diamminedichloro platinum (II) $Cl_2N_2H_6Pt$ M. W. 300.05
B: Carboplatin (CBDCA, JM-8, NSC241240) *cis*-diamminine-1, 1-cyclobutanedicarboxylate platinum (II) $C_6H_{12}N_2O_4Pt$ M. W. 371.26
C: 254-S (NSC375101 D) (Glycolato-O, O') diammine platinum (II) $C_2H_8N_2O_3Pt$ M. W. 303.2
D: DWA2114R (R)-1, 1-cyclobutanedicarboxylato-(2-aminomethyl-pyrrolidine)-platinum (II) $C_{11}H_{18}O_4N_2Pt \cdot H_2O$ M. W. 455.37
E: NK121 *cis*-l, 1-cyclobutane dicarboxylato-(2R)-2-methyl-1, 4-butane diammine platinum (II) $C_{11}H_{20}N_2O_4Pt$ M. W. 439.37

Tumor cell lines. Human lung cancer cell lines used in this study were three SCLC cell lines, SBC-1 (JCRB0816), SBC-2 (JCRB0817), and SBC-3 (JCRB0818); one adenocarcinoma cell line, ABC-1 (JCRB0815), one squamous cell carcinoma cell line, EBC-1 (JCRB0820), and one adriamycin-resistant SCLC subline, SBC-3/ADM_{30}. SBC-3 and EBC-1 were established from previously untreated patients, and the other lines came from patients previously treated by combination chemotherapy that did not include cisplatin. All the cell lines were maintained in RPMI-1640 medium (Grand Island Biological Co., Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) at 37°C in a humidified atomosphere with 5% carbon dioxide.

In vitro colony assay of cell lines. The culture system employed in this study, with some modifications, was first devised by Hamburger and Salmon (15). Briefly, single-cell suspensions (5×10^4 cells/ml) of each cell line in the exponential growth phase were exposed to the platinum analogs at 4 to 7 graded concentrations for 1h at 37°C, and then plated onto a feeder layer (15% FBS plus RPMI-1640 plus 0.5% agarose) as reported previously (16-17). Colonies were counted with an automated particle counter (CP-3000, Shiraimatsu Instruments Co., Osaka, Japan) after incubation for 14 days. Dose-response curves were drawn by calculating the ratio of the number of colonies surviving at each drug concentration to those in control plates. All experiments were carried out in duplicate and were repeated three times.

Fresh tumor specimens. Nineteen tumor specimens were obtained from patients with histologically proven lung cancer. Specimens were obtained by aspiration of malignant effusions or by excisional biopsy of the primary and/or metastatic lesions. Malignant effusions were collected into heparinized containers and the contaminating red blood cells were eliminated by the Ficoll-Conray specific gravity method. Solid tumors were minced into small fragments, which were subsequently processed for single-cells by enzymatic dissociation. Suspensions of 5×10^5 cells/ml were exposed to the platinum analogs for 1h at 37°C at concentrations of 1μM, 10μM, 100μM for cisplatin and 254-S, and at 10μM, 100μM, 1,000μM for carboplatin, DWA2114R, and NK121. Cells were washed twice and plated onto a feeder layer. Colonies were counted manually with an inverted microscope after incubation for 14 days.

Cross-resistance. The SBC-3/ADM_{30} subline was established by continuous exposure of the parental SBC-3 cells to increasing concentrations of adriamycin, followed by a cloning procedure. SBC-3/ADM_{30} cell were 30-fold more resistant to adriamycin than the parental SBC-3 cells, in terms of the 70% inhibitory concentration (IC_{70}) determined by colony assay. The cells also exhibited a pleiotropic type of drug resistance (19-20). The relative resistance of SBC-3/ADM_{30} cells to each of the platinum analogs was determined by dividing the IC_{70} value for the

resistant cells by that for the parental SBC-3 cells.

Analysis of antitumor activity. To assess the antitumor activity of the platinum analogs, the ratio of the maximum tolerated dose (MTD), for humans, of each drugs to the IC_{70} value determined by colony assay was calculated in each cell line tested. The antitumor activity appeared to elevate as the ratio increased. Friedman's two-way layout analysis of variance was applied in order to compare the antitumor activity of each platinum analog. The differences in antitumor activity were analyzed by Turkey's multiple comparison method. Kendall's coefficient of concordance (τ) was calculated to confirm the consistency of the ranking of antitumor activity for these platinum analogs. The MTD determined by phase I studies or recommended for phase II studies was 100 mg/m^2 (333 μmol/m^2) for cisplatin, 100 mg/m^2 (330 μmol/m^2) for 254-S, 360 mg/m^2 (819 μmol/m^2) for NK121, 400 mg/m^2 (1,077 μmol/m^2) for carboplatin, and 1,000 mg/m^2 (2,196 μmol/m^2) for DWA2114R.

In vitro combination. Single-cell suspensions were exposed to platinum analog alone, etoposide alone, and to both platinum analog and etoposide concurrently at graded concentrations for 1 h, then washed and plated onto a double-layer soft agarose system as mentioned above.

Analysis of drug combination effects. Dose-response curves of SBC-3 cells to each drug were fitted to a linear transformation of the median-effect equation determined by the method of Chou and Talalay (18).

$$\log (FC/(1 - FC)) = m \log (D) - m \log (Dm)$$

where FC is the fractional cytotoxicity, D is the drug dose, Dm is the median-effect dose that is required for 50% cytotoxicity (intercept of the X-axis), and m is a Hill-type coefficient. The synergism, additivity, or antagonism of the two drug effects was quantitatively determined by the combination index (CI). The CI is defined by

$$CI = \frac{(D)_1}{(Dx)_1} + \frac{(D)_2}{(Dx)_2} + k\frac{(D)_1 (D)_2}{(Dx)_1 (Dx)_2}$$

where Dx is the dose that is required to produce x% effect. CI $<$ 1 means synergism, CI $=$ 1 means additivity, and CI $>$ 1 means antagonism. This model of drug-drug interactions requires the drugs to have mutually exclusive or mutually non-exclusive mechanism of interaction. For mutually exclusive drugs, k is equal to 0, and for mutually non-exclusive drugs, the value of k is equal to 1. A mutually non-exclusive assumption makes the CI values slightly higher and prevents overestimation of synergism. Thus, in the current study, the interaction of the two drugs was assumed to be mutually non-exclusive. Graphs were plotted by calculating CI values as a function of FC. In addition, the area under the combination index curve (AUCI) was calculated to evaluate the overall effect of the various drug combinations. The AUCI value is equal to 1 when absolute additivity is present. AUCI values were calculated using Simpson's integration formula by dividing FC into 1,000 intervals. Calculations and the plottings of graphs were done automatically by a 32-bit personal computer (NEC PC-9801 RS).

Results

Antitumor activity against cell lines. All the platinum analogs showed a cytotoxic effect in a dose-dependent manner within the concentration ranges tested in the individual experiments. To evaluate and compare the antitumor activity of these platinum compounds, their MTD/IC_{70} ratios were calculated (Table 1). Taking these ratios as the ordered metric scale of antitumor activity, Friedman's test with replication was applied, and yielded the highly significant figure of 38.8 ($p = 7.5 \times 10^{-8}$), indicating that there were significant differences in antitumor activity among these five platinum analogs. The average ranking values were as follows: 13.5 for cisplatin, 8.9 for 254-S, 7.7 for DWA2114R, 5.8 for NK121, and 4.1 for carboplatin. When the antitumor activity was compared by Turkey's multiple comparison method, cisplatin was significantly superior to all the newly developed analogs, and 254-S was superior to carboplatin with a significance ($p = 0.0271$). No significant differences in the antitumor activity were seen mutually between carboplatin, DWA2114R, and NK121. The Kendall's τ value was 0.607 ($p < 0.01$), which confirmed the consistency of the ranking of the antitumor activity of these platinum analogs.

Antitumor activity against clinical specimens. The 19 clinical specimens tested consisted of 2 SCLCs, 12 adenocarcinomas, 3 squamous cell carcinomas, and 2 large cell carcinomas. Twelve of these specimens were from previously untreated patients, and the others were from patients previously treated with combination chemotherapy including cisplatin. In case of clinical specimens, if the maximum designated concentration of a drug provided under 70% cytotoxicity, it was regarded as IC_{70}, and if the minimum designated concentration provided over 70% cytotoxicity, it was also regarded as IC_{70}. The MTD/IC_{70} ratio of each drug except NK121 was calculated for all tumor specimens, and the antitumor activity of the platinum analogs was evaluated (Table 2). The average ranking values were 3.4 for 254-S, 2.9 for cisplatin, 1.9 for DWA2114R, and 1.7 for carboplatin (Friedman's test statistic = 23.8, $p = 7.5 \times 10^{-8}$). By Turkey's method, cisplatin and 254-S were significantly superior to carboplatin, and 254-S was superior to

Table 1 Comparison of the antitumor activity of platinum analogs against human lung cancer cell lines

Cell line	Plating efficiency[a] mean (%) ± SD	IC_{70} (μmol/L)				
		Cisplatin (A)	Carboplatin (B)	254-S (C)	DWA2114R (D)	NK121 (E)
SBC 1	1.4 ± 0.3	3.6 (92.6)[b]	63.7 (16.9)	10.9 (30.2)	97.7 (22.5)	33.0 (24.8)
		4.6 (71.6)	72.5 (14.9)	11.1 (29.8)	117.1 (18.8)	39.4 (20.8)
		3.3 (102.1)	51.0 (21.1)	8.7 (37.8)	72.2 (30.4)	24.7 (33.1)
SBC-2	3.0 ± 1.2	3.5 (94.5)	24.4 (44.2)	9.4 (35.2)	104.5 (21.0)	49.0 (16.7)
		8.4 (39.6)	59.7 (18.1)	18.7 (17.6)	73.7 (29.8)	30.0 (27.4)
		7.8 (42.5)	71.7 (15.0)	13.6 (24.2)	95.7 (23.0)	68.5 (12.0)
SBC-3	2.0 ± 0.8	2.4 (140.4)	87.9 (12.3)	11.7 (28.2)	109.7 (20.0)	20.0 (40.9)
		5.3 (63.0)	68.0 (15.8)	19.3 (17.1)	117.2 (18.7)	23.1 (35.4)
		6.8 (49.1)	75.5 (14.3)	11.3 (29.2)	106.9 (20.6)	27.2 (30.2)
ABC-1	2.8 ± 0.8	11.9 (28.0)	119.0 (9.1)	27.2 (12.2)	182.4 (12.0)	154.2 (5.3)
		10.2 (32.5)	164.4 (6.5)	15.6 (21.2)	128.9 (17.0)	165.7 (4.9)
		5.4 (62.0)	121.1 (8.9)	15.6 (21.2)	249.0 (8.8)	154.2 (5.3)
EBC-1	4.9 ± 1.9	43.9 (7.6)	666.6 (1.6)	55.1 (6.0)	681.7 (3.2)	407.0 (2.0)
		89.3 (3.7)	607.0 (1.8)	119.6 (2.8)	639.8 (3.4)	739.7 (1.1)
		119.1 (2.8)	546.8 (2.0)	150.3 (2.2)	735.9 (3.0)	324.6 (2.5)
Average of the ranks		13.5	4.1	8.9	7.7	5.8

a: determined from no. of colonies/no. of cells plated per 35-mm culture dish. *b:* numbers in parentheses are MTD/IC_{70} ratios (L/m²), which represent relative antitumor activity. The MTD of cisplatin, carboplatin, 254-S, DWA211R and NK121 is 333 μmol/m², 1,077 μmol/m², 330 μmol/m², 2,196 μmol/m² and 819 μmol/m², respectively. SD: standard deviation; IC_{70}: 70% inhibitory concentration; MTD: maximum tolerated dose. $p = 6.7 \times 10^{-8}$ (A *vs* B); $p = 0.0271$ (B *vs* C); $p = 0.0345$ (A *vs* C); $p = 0.0035$ (A *vs* D); $p = 2.2 \times 10^{-5}$ (A *vs* E). Probability values were estimated by Turkey's multiple comparison method.

Table 2 Comparison of the antitumor activity of platinum analogs against clinical tumor specimens

Case no.	Histology	Prior CT[a]	Source material	Plating efficiency[b] (%)	MTD (μmol/m²)/IC_{70} (μmol/L)				
					Cisplatin (A)	Carboplatin (B)	254-S (C)	DWA2114R (D)	NK121
1	Sm	−	P.E.	0.0078	55.5	13.5	329.8	2.2	ND
2	Sm	+	S.M.	0.1180	3.3	1.1	3.3	2.2	ND
3	Ad	−	P.E.	0.0100	83.3	33.7	109.9	22.0	ND
4	Ad	−	P.E.	0.0864	14.5	4.7	22.0	2.2	ND
5	Ad	+	P.E.	0.0460	66.7	1.1	47.1	2.2	ND
6	Ad	+	P.E.	0.0940	3.3	1.1	7.3	2.2	ND
7	Ad	−	P.T.	0.0102	3.3	3.6	164.9	2.2	ND
8	Ad	−	P.E.	0.0320	111.1	107.7	329.8	219.6	3.2
9	Ad	+	P.E.	0.0318	3.3	10.8	3.3	219.6	81.9
10	Ad	−	P.T.	0.0136	3.3	1.1	3.3	2.2	0.8
11	Ad	−	P.E.	0.0060	111.1	107.7	329.8	11.0	63.0
12	Ad	+	P.E.	0.0224	3.3	1.1	3.3	2.2	0.8
13	Ad	+	C.E.	0.0720	3.3	1.5	3.3	6.1	2.4
14	Ad	+	P.E.	0.0075	14.5	13.5	8.2	73.2	81.9
15	Sq	−	P.T.	0.0300	333.3	1.1	329.8	2.2	ND
16	Sq	−	P.T.	0.0077	6.7	3.6	20.6	2.2	0.8
17	Sq	−	P.T.	0.0074	9.5	107.7	109.9	2.2	8.2
18	La	−	P.E.	0.0860	13.3	8.3	164.9	2.2	ND
19	La	−	L.M.	0.0136	3.3	1.1	3.3	2.2	0.8
Average of the ranks					2.9	1.7	3.4	1.9	

a: prior chemotherapy including cisplatin. *b:* determined from no. of colonies/no. of cells plated per 35-mm culture dish. MTD: maximum tolerated dose; IC_{70}: 70% inhibitory concentration; ND: not done; Sm: small cell carcinoma; Ad: adenocarcinoma; Sq: squamous cell carcinoma; La: large cell carcinoma; P.E.: pleural effusion; S.M.: skin metastasis; P.T.: primary tumor; C.E.: pericardial effusion; L.M.: lymph node metastasis. $p = 0.0117$ (A *vs* B); $p = 0.001$ (B *vs* C); $p = 0.0020$ (C *vs* D). Probability values were estimated by Turkey's multiple comparison method.

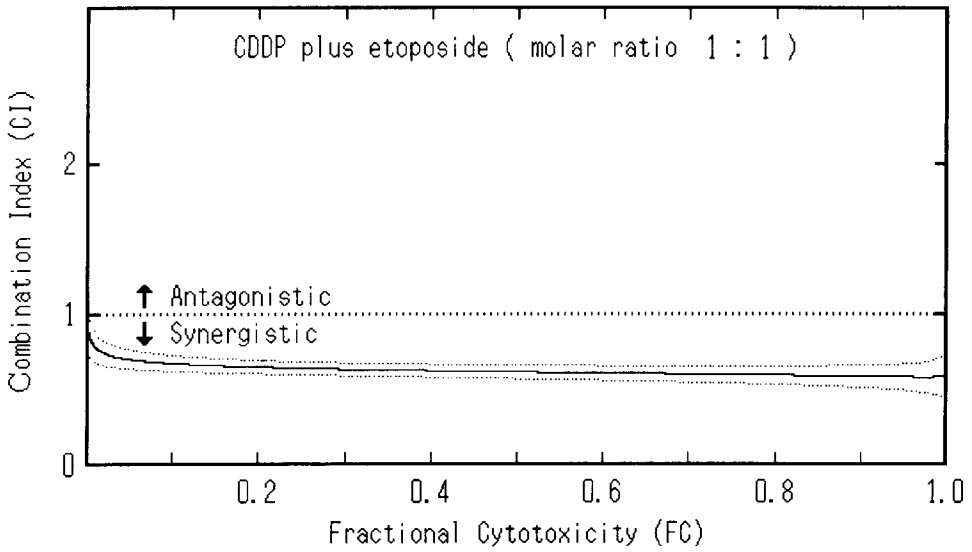

Fig. 2 Computer simulation of the combination index (CI) as a function of fractional cytotoxicity (FC). The middle solid curve indicates the mean CI of four experiments; upper and lower dotted curves indicate mean CI ± standard error, respectively. The area below a CI of 1 represents a synergistic interaction; the area above a CI of 1 represents an antagonistic interaction. As for the combination of cisplatin plus etoposide at a fixed molar ratio of 1:1, the CI values were below 1 over the entire range of FC values, suggesting synergitic interaction.

Table 3 Comparison of the IC_{70} values of platinum analogs for SBC-3 and SBC-3/ADM_{30} cells.

Platinum analog	Number of experiments	Mean IC_{70} (μmol/L) ± SD		Relative resistance[a]	p-value[b]
		SBC-3	SBC-3/ADM_{30}		
Cisplatin	4	3.9 ± 1.7	6.7 ± 2.7	1.7	0.091
Carboplatin	4	76.0 ± 20.7	126.3 ± 66.6	1.7	0.135
254 S	6	19.2 ± 9.3	9.5 ± 5.4	0.5	0.010[c]
DWA2114R	4	87.3 ± 35.5	159.9 ± 71.4	1.8	0.057
NK121	3	22.6 ± 2.4	91.1 ± 25.8	4.0	0.026[d]
Plating efficiency (%)		2.6 ± 0.9	3.5 ± 1.6		

a: determined by dividing the IC_{70} for the resistant SBC-3/ADM_{30} cells by the IC_{70} for the sensitive SBC-3 cellls. *b:* probability values estimated by Student's paired t-test (one-tailed). *c:* higher mean IC_{70} value for SBC-3 cells than for SBC-3/ADM_{30} cells. *d:* higher mean IC_{70} value for SBC-3/ADM_{30} cells than for SBC-3 cells. IC_{70}: 70% inhibitory concentration; SD: standard deviation

DWA2114R. Kendall's τ value was 0.417, which was also significant ($p < 0.01$).

Cross-resistance patterns to adriamycin. SBC-3/ADM_{30} cells showed a 1.7-fold increase in resistance to cisplatin and carboplatin, a 4.0-fold increase for NK121, and a 1.8-fold increase for DWA2114R, whereas they were 2.0-fold more sensitive to 254-S (Table 3).

Combination of cisplatin and etoposide. Synergism was reproducibly observed when cisplatin and etoposide were at a fixed molar ratio 1:1 (Fig. 2). The mean CI value was below 1 over the entire range of FC values. The mean AUCI in four experiments was equal to 0.62 (Table 4), which indicated a consistent synergistic effect in the combination.

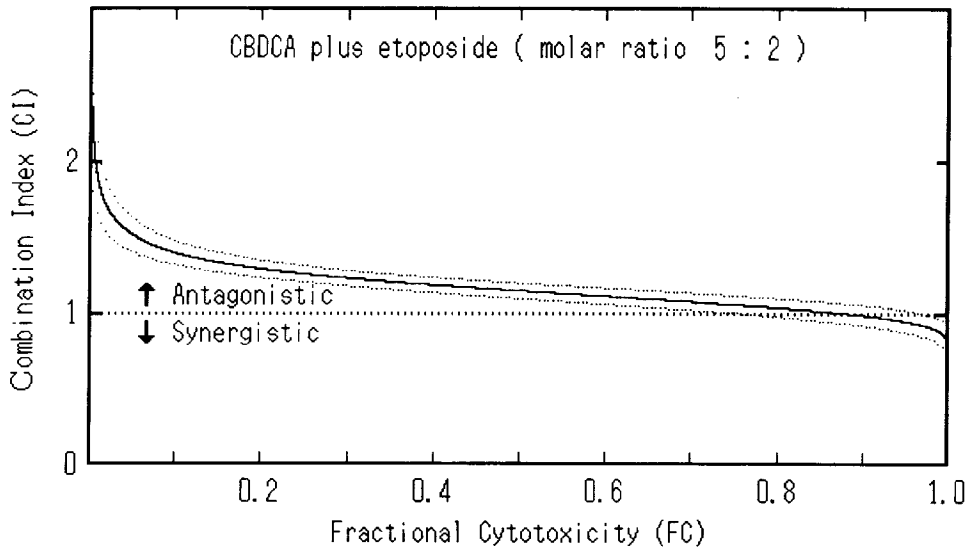

Fig. 3 Graphical presentation of the CI for the combination of carboplatin plus etoposide at molar ratio of 5:2. The middle solid curve indicates the mean CI of five experiments; upper and lower dotted curves indicate mean CI ± standard error, respectively. The results indicated that there was a moderate antagonism at low FC values and close to additive effect at higher FC values.

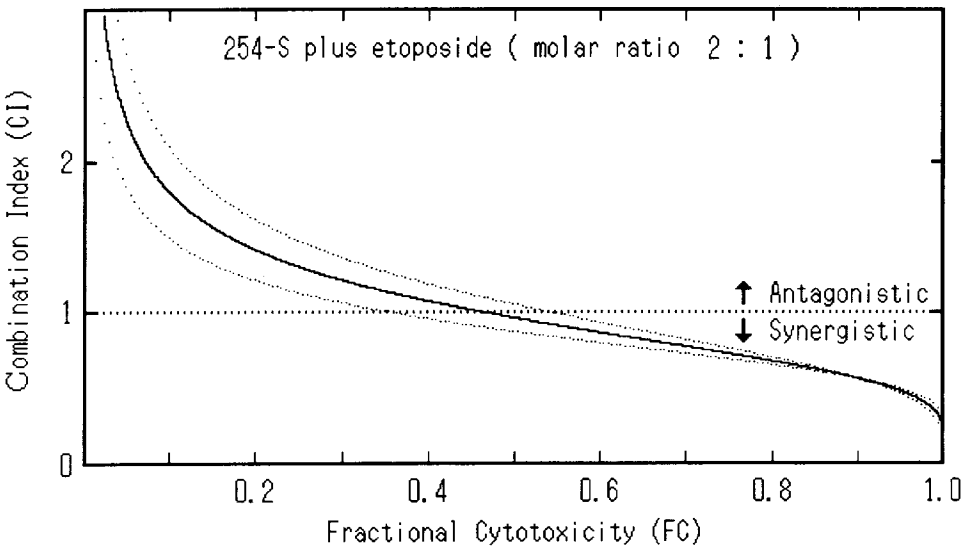

Fig. 4 Graphical presentation of the CI for the combination of 254-S plus etoposide at molar ratio of 2:1. The middle solid curve indicates the mean CI of three experiments; upper and lower dotted curves indicate mean CI ± standard error, respectively. An antagonism was observed at low FC values, whereas a marked synergism was obtained at higher FC values. Note that the mean CI values at FC greater than 0.9 were lower than those for the combination of cisplatin plus etoposide.

Combination of carboplatin and etoposide. An additive effect was noted in the combination of carboplatin and etoposide at a molar ratio 5:2. The mean CI curve gradually decreased as the FC value increased (Fig. 3).

Table 4 Combination index (CI) as a function of fractional cytotoxicity (FC) and the area under the combination index curve (AUCI)

Drug combination	CI_{10}[a]	CI_{30}	CI_{50}	CI_{70}	CI_{90}	AUCI
CDDP:ETP (1:1)[b]	0.67 ± 0.06[c]	0.63 ± 0.05	0.61 ± 0.05	0.60 ± 0.06	0.58 ± 0.09	0.62 ± 0.05
CBDCA:ETP (5:2)	1.40 ± 0.09	1.23 ± 0.06	1.14 ± 0.06	1.08 ± 0.06	0.99 ± 0.07	1.18 ± 0.05
254-S:ETP (2:1)	1.78 ± 0.37	1.21 ± 0.18	0.96 ± 0.11	0.77 ± 0.06	0.56 ± 0.01	1.11 ± 0.16

a: CIx represents the combination index at X% cell lethality, *b:* molar ratio, *c:* mean ± standard error
ETP: etoposide; CDDP: cisplatin; CBDCA: carboplatin.

The mean CI curve was below 1 only when FC was greater than 0.9, so this combination was estimated to be less efficacious than the cisplatin and etoposide combination. The AUCI was 1.18, when suggested an additive or subadditive interaction between carboplatin and etoposide.

Combination of 254-S and etoposide. The combination of 254-S and etoposide at a molar ratio of 2:1 produced an antagonism at low FC values, whereas a marked synergism was obtained at higher FC values (Fig. 4). This result indicated that a large dose would increase the synergistic interaction of these two drugs.

Discussion

The discovery of cisplatin was an epoch-making development in the history of cancer chemotherapy and any recent advances of chemotherapy are mainly due to this drug. However, the toxic side effects of cisplatin often restrict its clinical use. Numerous platinum analogs have been enthusiastically synthesized in search for alternative active compounds with a reduced level of toxicity. In consequence of preclinical studies using animal tumor models, four platinum derivatives which were more active and less toxic than cisplatin have been selected. If one could predict the clinical response of a certain specified tumor to a new agent using human cancer cells before phase II clinical trials, such risky trials could be minimized in the future. The present study attempted to evaluate the antitumor activity of several newly developed platinum analogs by an *in vitro* colony assay using human lung cancer cell lines and clinical tumor specimens.

When predicting a likely clinical response to a certain cytotoxic agent on the basis of *in vitro* drug sensitivity testing, the ratio of the peak plasma concentration (PPC) *in vivo* to the IC_{50-90} *in vitro* or the ratio of the area under the concentration-time curve (AUC) *in vivo* to the IC_{50-90} *in vitro*, are often used as a therapeutic index. In the case of platinum analogs, however, the pharmacokinetic behavior of cisplatin is quite different from that of the "second generation" platinums, especially with regard to its protein binding capacity (19-21). Approximately 90% of an administered dose of cisplatin binds tightly to serum protein and only the non-protein-bound platinum exerts its cytotoxic effect. In contrast, the second generation platinum analogs stay mainly free in the serum (22-24). Therefore, the AUC of non-protein-bound component of the second generation platinum compounds is generally large, while that of cisplatin remains quite small. Because new drugs often lack precise data of pharmacokinetics, it is sometimes difficult to compare the antitumor activity of cisplatin and other platinum analogs by using the PPC/IC ratio and/or the AUC/IC ratio. We used the MTD/IC_{70} ratio in this study because a clinical dosage of platinum analogs is restricted only by the MTD and is not related to the pharmacokinetics or to the accumulation of these drugs in organs, and the MTD can be preclinically estimated to some extent by the animal scale-up method (25). Non-parametric statistical analysis was applied to compare the antitumor activity of the platinum analogs. Regarding lung cancer cell lines, cisplatin was the most potent of the platinum analogs tested, and 254-S was more potent than carboplatin. In the clinical specimens, cisplatin was more potent than carboplatin, and 254-S was more potent than both carboplatin and DWA2114R. There were no inconsistencies in the order of antitumor activity shown by the platinum analogs between the cell lines and the clinical specimens, indicating that 254-S was the most potent drug among the newly developed platinum analogs.

All three SCLC cell lines were less sensitive to carboplatin than to cisplatin, which contradicts the recent clinical preference for carboplatin. It is possible that carboplatin exerts its effect in a time-dependent manner and perhaps, the 1-h exposure time was too short to exert fully its intrinsic antitumor activity in our colony assay.

SBC-3/ADM_{30} cells showed a pattern of pleiotropic

drug resistance as reported previously (26), but the cells sustained a considerable sensitivity to the second-generation platinums. Above all, it is noteworthy that the cells were 2.0-fold more sensitive to 254-S than their parent SBC-3 cells were. To our knowledge, there have been only a few reports of a collateral sensitivity in anthracycline-resistant cell lines (27-28).

Although cisplatin monotherapy produces only a 10-15% response rate (29) and etoposide produces a 40-60% response rate (30) in SCLC, the response rate of cisplatin plus etoposide is from 70% to almost 100% (31). In 1979, Schabel *et al.* demonstrated synergism between cisplatin and etoposide in a bioassay using murine P388 leukemia (32). Thereafter, this combination has been widely used not only for SCLC but also for non-SCLC patients (33). However, Schabel's model could not reflect the specificity of the combination for human tumors. That is why we used human tumor cells to quantify the effects of various 2-drug combinations. Consistent synergism was demonstrated for cisplatin plus etoposide over the whole range of FC values. This result correlated well with previous clinical experience.

The clinical activity of a carboplatin and etoposide combination *in vivo* cannot be fully interpreted, because carboplatin has only recently been introduced into clinical use. However, a few reports are available. Bishop *et al.* (34) have reported that the combination was equally active and less toxic than the cisplatin and etoposide combination in previously untreated patients with SCLC. Smith *et al.* (35) reported that response rate of the combination was comparable to the cisplatin and etoposide combination, but that the response duration and the survival time were disappointing. Our current study indicated that the combination was not superior to the cisplatin and etoposide combination. In contrast, marked synergism was obtained at higher concentrations of the 254-S and etoposide, but antagonism occurred at low concentrations.

To find out more active combinations, disease-oriented *in vitro* simulation studies which correctly predict a clinical response will become increasingly popular. Researchers may anticipate the need to establish drug sensitive cell lines like SBC-3, which is sensitive to a wide variety of drugs within clinically achievable concentrations in humans. If several drug sensitive cell lines were available, a rational combination with an optimal ratio of antitumor agents could be realized. Because the combination index used in this study can theoretically quantify the combined effects of three or more drugs, further realistic simulation studies of drug administration in humans should be possible.

In summary, our data indicate that 254-S is the most promising one among new platinum analogs not only for monotherapy but also for combination with etoposide, and that it may have some potential to overcome resistance to adriamycin by virtue of collateral sensitivity.

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