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Prediction of the antitumor activity of new platinum analogs based on their ex vivo pharmacodynamics as determined by bioassay*

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Summary. We report the predictive model for the clinical response of new platinum analogs against lung cancer by a bioassay using human lung-cancer cell lines including small-cell (SCLC) and non-small-cell lung cancer (NSCLC). Exponentially growing cells of six different SCLC and six NSCLC lines were exposed to different concentrations of the three platinum compounds, cisplatin, carboplatin, and 254-S in a double-agar colony-forming cell assay. The concentrations inhibiting 50% of colony formation (IC_{50} value) for cisplatin, carboplatin and 254-S in SCLC cell lines were significantly lower than those in NSCLC cell lines. A total of 15 patients entered the pharmacological study. In all, 80 mg/m^2 cisplatin, 450 mg/m^2 carboplatin, and 100 mg/m^2 254-S were each given to five patients by intravenous drip infusion. Bioassay as well as chemical assay was achieved by clonogenic techniques using NCI-H-69 (SCLC cell line) and PC-9 (NSCLC cell line) as target cells. Biological comparison of antitumor activity was performed on the basis of the antitumor activity of patients' plasma using the antitumor index (ATI), which was defined as the area under the percentage of colony suppression versus time curve obtained by bioassay and calculated by the trapezoidal rule. When NCI-H-69 and PC-9 were used as target cells for bioassay, colony-inhibitory activity was revealed by the ATIs. The ATIs obtained by bioassay showed better correlation than the AUCs obtained by chemical assay with the clinical response for cisplatin and carboplatin against SCLC and NSCLC, according to the following equation: [Reported Response (%)] = 11.5668+0.0014 × [ATI] (r = 0.97). The response rates for 254-S against SCLC and NSCLC were predicted by this formula to be 40%–65% and 14%–16%, respectively. 254-S is prospectively suspected of having the same, if not more, activity then carboplatin against SCLC and of having almost the same activity as cisplatin against NSCLC.

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Introduction

Lung cancer is one of the most common neoplastic diseases in Japan. The development of additional active anticancer agents is essential for the improvement of lung cancer treatment because the majority of patients die of disseminated disease. The chemotherapeutic strategy for lung cancer differs between small-cell (SCLC) and non-small-cell lung cancer (NSCLC). SCLC is more responsive than NSCLC to chemotherapy, with 15%–20% of patients surviving for >3 years after treatment using the nonsurgical approach. Although many kinds of anticancer agents have been tried in the treatment of NSCLC, this disease is known to be a chemo-resistant tumor for which no standard chemotherapy has been identified. The development of new anticancer agents that are effective against SCLC as well as NSCLC is necessary to improve the results of lung cancer treatment.

cis-Diamminedichloroplatinum(II) (cisplatin, CDDP) is an agent [24] that is currently used in treating a wide variety of tumor types, including testicular [12, 40], ovarian [3, 43], bladder [35, 44], and lung cancers [13, 14, 32]. However, considerable renal toxicity, nausea and vomiting, and neurotoxicity are serious dose-limiting problems complicating its clinical use. In an attempt to improve the clinical use of the platinum complex, a number of analogs have been developed that do not produce nephrotoxic effects but have antitumor activity equivalent or superior to that of cisplatin.

cis-Diammine-1,1-cyclobutanedicarboxylateplatinum-(II) (carboplatin, CBDCA) and *cis*-diammine(glycolato)-platinum (NSC 375101D, 254-S) are second-generation platinum-coordination complexes that are less nephrotoxic and have retained higher antitumor activity than cisplatin

Table 1. Characteristic of human lung-cancer cell lines

Cell line	Histological type	Source	Prior treatment	Plating efficiency (%)
NCC-c-Lu-134	C-SCLC	L	No.	0.04
NCC-c-Lu-135	C-SCLC	L	No	0.04
NCC-c-Lu-139	C-SCLC	L	No	0.03
NCI-H-69	C-SCLC	PE	Yes[a]	1.09
NCI-N-231	C-SCLC	L	No	0.08
NCI-N-857/N-230	C-SCLC	L	No	0.09
PC-1	Squamous	LN	No	1.44
PC-3	Squamous	LN	Yes[a]	1.34
PC-7	Adeno	LN	No	0.77
PC-9	Adeno	LN	No	1.62
PC-13	Large	L	No	1.49
PC-14	Adeno	L	No	0.60

[a] NCI-H 69 and PC 3 were established from patients treated by chemotherapy without cisplatin
L, Lung; PE, pleural effusion; LN, lymph node; C, classic; SCLC, small-cell lung cancer; Squamous, squamous-cell carcinoma; Adeno, adenocarcinoma of the lung; Large, large-cell carcinoma

in some animal models. Carboplatin has been found to show activity against a variety of experimental tumor systems, including P388, L1210, B16 melanoma, and colon 26 carcinoma [41, 42]. 254-S has also exhibited anticancer activity superior to that of cisplatin against P388, colon tumor 38, Lewis lung tumor, B16melanoma, Walker 256 carcinosarcoma, MX-1 breast tumor, and Burkitt's lymphoma (DAUDI) in preclinical studies [33].

Many platinum analogs, including carboplatin and 254-S, have indeed been synthesized, but it has been difficult to predict whether these analogs will show superior antitumor activity against human cancers as compared with their parent compound cisplatin before clinical phase II and III trials have been completed. In addition to the preclinical in vitro activity, the factors that can also influence chemotherapeutic effects include the peak achievable plasma concentration and the pharmacological behavior of the investigational drug as well as the chemosensitivity of the target cancer cells. These factors should be considered in comparisons of the anticancer activity of new agents vs their parent compounds.

The objective of the present study was to establish a new predictive model for the clinical response of new platinum analogs against lung cancer by a bioassay using human lung-cancer cell lines including SCLC and NSCLC. The biological pharmacology of the active state of platinum, which was determined by ex vivo pharmacodynamics, was determined by investigating the colony-inhibitory activity of the plasma of patients who were receiving platinum compounds. Finally, a prospective evaluation of the antitumor activity of an investigational new platinum, 254-S, by this screening model was carried out.

Materials and methods

Cell lines. Studies were performed using 12 different established human lung-cancer cell lines. The three SCLC cell lines NCC-c-Lu-134, NCC-c-Lu-135, and NCC-C-Lu-139 were established at the Pathology Division of the National Cancer Center Research Institute [39] and were supplied by Dr. Shimosato; the three other SCLC cell lines NCI-H-6, NCI-N-231, and NCI-N-857/N-230 were kindly provided by Dr. Minna [5], National Cancer Institute-Navy Medical Oncology Branch (USA). PC-1, PC-3, PC-7, PC-9, PC-13, and PC-14 were derived from human NSCLC (adenocarcinoma, squamous-cell carcinoma, and large-cell carcinoma of the lung) [16] and were provided by Prof. Hayata, Tokyo Medical College. The main characteristics of these cell lines are summarized in Table 1. The cells were propagated in 75-cm^2 plastic flasks (Corning Glass Works, corning, N. Y.) in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 100 µg/ml streptomycin and 100 IU/ml penicillin in an incubator at 37°C under a humidified atmosphere containing 5% CO_2 and 95% air. All reagents for culture were obtained from Grand Island Biological Co. (Grand Island, N. Y.).

Drugs. In addition to the reference drug cisplatin, carboplatin and 254-S were used for both an in vitro sensitivity test and a clinical pharmacology study. Cisplatin and carboplatin were generously donated by Bristol-Meyers Research Institute (Tokyo, Japan), and 254-S is an investigational agent that was synthesized and donated by the Shionogi Pharmaceutical Company (Osaka, Japan). All of the platinum compounds used in the course of the study were standard patient-treatment formulations. Solutions of the agents were prepared in RPMI 1640 medium containing 10% FCS just before in vitro analysis. For clinical use, each agent was diluted in 5% glucose solution before its administration to patients.

In vitro chemosensitivity against lung-cancer cell lines. The colony-forming assay devised by Hamburger and Salmon [15, 26] was modified and used for evaluation of the in vitro anticancer activity of the three platinum analogs against the human lung-cancer cell lines. Briefly, the cells to be tested were harvested from cell-culture flasks and then washed with RPMI 1640 medium. The cells were made into single-cell suspensions by mechanical disociation and were counted using a Coulter Counter Model ZB1 (Coulter Electronics, Inc., Fla.). Viability of the tumor cells was confirmed by trypan blue dye-exclusion test (>90% of cell viability). In all, 1 ml tumor-cell suspension (1×10^5 cells/ml of the PC series and NCI-H-69 and 3×10^5 cells/ml of the other SCLC cell lines) in 10% FCS and 0.5% Bacto-Agar (Difco Laboratories, Mich.) containing RPMI 1640 medium with or without the appropriate concentration of the platinum compounds was pipetted onto 1 ml substrate in a $3.5 \times$ 1-cm tissue-culture multiwell plate (Linbro; Flor Laboratories, Inc., Va.). The substrate contained 0.5% agar in enriched McCoy's 5A medium that consisted of 400 ml McCoy's 5A medium (Grand Island Biological Company. N. Y.), 40 ml 10% heat-inactivated FCS, 20 ml 5% heat-inactivated horse serum (Grand Island Biological company, N. Y.), 4 ml 2.2% sodium pyruvate, 4 ml 200 mM glutamine, and 0.8 ml 2.1% serine (Wako Pure Chemical Industry, Osaka, Japan). After the tumor cells had been plated, they were inspected under an inverted microscope to confirm that each cell had separated into single cells in the upper layer without forming tumor clumps in the dishes; the cells were then incubat-

ed at 37°C in a highly humidified incubator containing 5% CO_2 for 9–21 days (until maximal colony growth on control plates of each cell line was obtained). The target tumor cells were continuously exposed to different concentrations of each agent (0.1–100 μg/ml) in soft agar. Each test was performed in triplicate. After 9–21 days of incubation, colonies that were >60 μm in diameter were counted by an automatic particle counter (CP-2000, Shiraimatsu Instrument, Osaka, Japan). The percentage of colony survival was calculated using the following formula:

$$100 \times \frac{\text{Mean counts in test dishes}}{\text{Mean colony counts in three control dishes}}.$$

The drug sensitivity of the lung-cancer cell lines was compared by the IC_{50} (drug concentration achieving 50% colony inhibition) values that were determined graphically after a dose-response curve had been obtained for each cell line.

Patients and sample collection. A total of 15 patients were entered in the pharmacology study. To be eligible for this study, patients with lung cancer or other malignancies had to have failed conventional chemotherapy or have no available effective chemotherapy. No patient had previously received platinum compounds. A performance status of 0, 1, or 2 on the Eastern Cooperative Oncology Group scale was needed. Adequate bone marrow function (WBC counts, >3,000/μl, platelet counts, >100,000/μl), liver function (bilirubin, <2 mg/dl), and renal function (serum creatinine <1.5 mg/ml) were required before chemotherapy. Informed consent was obtained from all patients before treatment.

The doses of cisplatin, carboplatin, and 254-S were 80, 450, and 100 mg/m^2, respectively; the 254-S dose was that recommended for phase II study [2]. These platinum compounds were given to five patients each by intravenous (i.v.) drip infusion over 30 min in 150 ml 5% glucose solution without diuretic agents. The patients who received cisplatin also received 3,000 ml normal saline before and after administration of the drug. An indwelling i.v. cannula was placed in the arm opposite to that receiving the drug. Blood samples were obtained before and just after the end of the drug infusion and at 5, 15, 30, 60, 120, 240 and 480 min thereafter. Blood was collected by a hepatin-containing syringe and plasma was immediately separated by centrifugation at 600 *g* for 10 min. As soon as plasma had been prepared, part of it was passed through an Amicon CF 25 filter (Amicon Corporation, Danvers, Mass.) by centrifugation at 2,000 *g* for 30 min at 4°C to remove protein. The protein-free ultrafiltrate for chemical assay and aliquots of whole plasma for chemical assay and bioassay were stored at –70°C until analysis.

Pharmacokinetic analysis. Total and ultrafilterable platinum in the plasma were assayed [20] using a Hitachi model 170-50A flameless atomic absorption spectrometer (Hitachi, Tokyo, Japan) according to Takahashi et al. [36]. The pharmacokinetic models were decided upon after inspection of each time-concentration point in a semilogarithmic plot. Following the end of the infusion a biexponential equation was fitted to the plasma platinum levels:

$$C = Ae^{-at} + Be^{-bt},$$

where C represents the concentration at time t and A, B and a, b represent the concentration and rate constants, respectively. The free platinum level of cisplatin was fitted to a monoexponential equation. These pharmacokinetic parameters were calculated using a computerized nonlinear least-squares technique by the computer program MULTI [45]. Concentration times time ($C \times T$) for the curve of platinum in plasma was estimated by calculating the AUC and AUCs until 510 min after the beginning of drug infusion were determined by the trapezoidal rule.

Detection of ex vivo pharmacodynamics. The biological activity of platinum was determined by clonogenic assay using PC-9 and NCI-H-69 cell lines as target cells. The details of the method for bioassay have been presented elsewhere [27]. In brief, 1×10^5 PC-9 cells or 3×10^5 NCI-H-69 cells were incubated with whole plasma from the patients for 1 h at 37°C in a humidified atmosphere containing 5% CO_2. After being washed twice with RPMI-1640 medium, they were plated on the substrate consisting of double-enriched McCoy's 5A medium with 0.5% agar. The plates were incubated for 9 and 14 days for PC-9 and NCI-H-69 cells, respectively, and the number of colonies were counted by an automatic colony counter. Colony suppression rates were calculated by comparing the numbers of colonies formed in plasma obtained from patients treated with platinum compounds with those formed in plasma before the administration of platinum compounds.

Comparison of the antitumor activity of the three platinum compounds based on their ex vivo pharmacodynamics. Biological comparison of antitumor activity was performed on the basis of the suppressive effect on colony formation in the patients' plasma. That is, curve for the percentage of colony suppression induced in the patient's plasma from just before treatment to 510 min after the beginning of chemotherapy was plotted. The area under the percentage of colony suppression vs time curve obtained for each platinum agent was calculated by means of the trapezoidal rule from before treatment to 510 min after the beginning of drug infusion using the same MULTI program used for the calculation of AUCs and was defined as the antitumor index (ATI). When NCI-H-69 (SCLC cell line) and PC-9 (NSCLC cell line) were used as target cells for the bioassay, the colony-inhibitory activity was revealed by the ATIs. The antitumor activity of the three platinum compounds was compared according to the ATIs on a biological pharmacology basis.

Statistical analysis. The difference in chemosensitivity between SCLC and NSCLC cell lines against three platinum compounds was compared by IC_{50} values and analyzed using Student's *t*-test. The correlation between the ATIs and the cumulative clinical response rates obtained for cisplatin and carboplatin against SCLC and NSCLC in phase II studies was processed and plotted by a simple least-squares regression method of the MYSTAT program (SYSTAT, Inc., Evanston, Ill.) using a Macintosh microcomputer.

Results

In vitro cytotoxicity of the platinum compounds against lung-cancer cell lines

The in vitro anticancer activity of cisplatin, carboplatin, and 254-S against six SCLC and six NSCLC cell lines was compared with the IC_{50} values. The plating efficiency of these cell lines in soft agar varied from 0.03% to 1.62%. NCI-H-69 and PC-9 exhibited the highest plating efficiencies among SCLC and NSCLC cell lines, respectively (Table 1). Each of the cell lines was tested with the three platinum compounds in a series of continuous drug-exposure experiments. The IC_{50}s for carboplatin were significantly higher than those for cisplatin and 254-S in both SCLC and NSCLC cell lines. No significant differences in IC_{50}s were detected between cisplatin and 254-S (Table 2). The IC_{50} values for cisplatin, carboplatin, and 254-S in SCLC cell lines were significantly lower than those in NSCLC cell lines (Table 2).

Pharmacokinetics of the platinum compounds as determined by the atomic absorption method

Plasma concentrations of total platinum for the three platinum compounds declined in a biexponential fashion. Ultrafilterable platinum in plasma (non-protein-bound active platinum concentration) declined biexponentially for carboplatin and 254-S, whereas the free platinum of cisplatin fitted to a monoexponential equation (data not shown). The peak plasma concentration was highest for carboplatin, followed by 254-S and cisplatin. AUCs for free platinum

Table 2. Differences in chemosensitivity between SCLC and NSCLC cell lines according to mean IC_{50} values

Cell line	Cisplatin (μg/ml)	Carboplatin (μg/ml)	254-S (μg/ml)
SCLC:			
NCC-c-Lu 134	0.27	0.8	0.27
NCC-c-Lu 135	0.25	0.9	0.27
NCC-c-Lu 139	0.2	0.7	0.33
NCI-H-69	0.07	0.9	0.08
NCI-N-231	0.09	0.34	0.25
NCI-N-857/N-230	0.25	1.45	0.29
Mean ±SD	0.18±0.08*	0.84±0.31**	0.26±0.09***
NSCLC:			
PC-1	0.85	4.33	0.84
PC-3	0.5	4.41	0.90
PC-7	1.8	4.42	1.90
PC-9	0.95	4.8	0.85
PC-13	0.9	5.42	1.20
PC-14	1.6	5.63	1.75
Mean ±SD	1.1±0.45*	4.9±0.48**	1.13±0.4***

Mean IC_{50} values for human lung-cancer cell lines were determined from the survival curves of three replicate colony assays and were compared between SCLC and NSCLC using Students *t*-test * $P = 0.0063$, ** $P = 0.0001$, *** $P = 0.0023$

as an active compartment were 3,446, 959, and 208 μg $min^{-1} \cdot ml$ for carboplatin, 254-S, and cisplatin, respectively, and those for total platinum were also highest for carboplatin (Table 3). A precise pharmacokinetic comparison of the three compounds has been presented elsewhere [28].

Comparison of the ex vivo pharmacodynamics of the platinum derivatives

The in vitro anticancer activity of the three platinum compounds was compared by bioassay. Target cell lines used in this study were NCI-H-69 and PC-9, representing SCLC and NSCLC, respectively. These two cell lines were selected because both were easily separated into single-cell suspensions by pipetting and they showed high plating efficiencies, forming >1,000 colonies on control plates. No significant differences were detected in the chemosensitivity of SCLC or NSCLC cell lines against cisplatin, carboplatin, or 254-S. Our previous study indicated good reproducibility for this bioassay system [27].

Percentage of colony suppression vs time curves in plasma from 0 to 510 min after the beginning of drug administration are plotted in Fig. 1 and listed in Table 3. When PC-9 was chosen as the target cell, antitumor activity could not be detected after 120 min and maximal colony suppression did not reach 50%. The ATIs shown in Table 3 revealed no statistical difference in the ATI/NSCLCs for cisplatin vs carboplatin and for cisplatin vs 254-S. However, 254-S had a significantly higher ATI/NSCLC value than did carboplatin.

When NCI-H-69 was used as the target cell, the peak percentage of colony suppression reached >75% for carboplatin and 254-S, and colony-suppressive activity for both agents was detected even after 510 min. However, anticancer activity induced by cisplatin disappeared in a monophasic pattern within 150 min. The ATI/SCLCs for carboplatin and 254-S were significantly higher than that of cisplatin (Table 3). These ATI data suggested that 254-S would have almost equivalent, if not higher, antitumor activity against SCLC than carboplatin.

Correlation between the ATIs and the clinical responses for cisplatin and carboplatin and prospective evaluation of the clinical response rate for 254-S in lung cancer

The response rates reported in phase II studies of cisplatin and carboplatin in lung cancer patients are summarized in Table 4. Although both cisplatin and carboplatin were active against SCLC, carboplatin showed a higher response rate than cisplatin when used as a single agent. However, carboplatin showed only marginal activity against NSCLC despite its higher AUC values for total and free platinum among the three platinum compounds. Cisplatin has been reported to be more active against NSCLC than carboplatin, although its AUCs for total and free platinum were lower than those of carboplatin.

The correlation between the ATIs and the clinical response for cisplatin and carboplatin was plotted in Fig. 2; it indicated significantly high correlation between these two factors when calculated using the equation [Reported Response (%)] = 11.5668 + 0.0014 × [ATI] ($r = 0.97$). However, because of the small number of points and the considerable variation in the SDs and confidence intervals in

Table 3. Comparison of three platinum compounds by antitumor index

Drugs	ATI/SCLC	ATI/NSCLC	AUC, free platinum (μg ml^{-1} min)	AUC, total platinum (μg ml^{-1} min)
Cisplatin	3,202±664	1,634±667	208	1,545
Carboplatin	19,004±5,436[a]	905±405	3,446	4,337
254-S	29,857±8,837[a]	2,377±461[b]	959	1,144

Biological comparison of antitumor activity was performed on the basis of the antitumor activity of patients' plasma using the antitumor index (ATI), which was defined as the area under the percentage of colony suppression versus time curve before treatment to 510 min after the beginning of chemotherapy, obtained by bioassay and calculated by means of the trapezoidal rule. ATI/SCLC and ATI/NSCLC indicated ATIs when NCI-H-69 and PC-9 were used as the respective target cells of the bioassay. AUCs before treatment to 510 min after the beginning of chemotherapy were determined from the computer-generated fit by the trapezoidal rule

[a] Significantly higher than the ATI/SCLC of cisplatin ($P < 0.05$)

[b] Significantly higher than the ATI/NSCLC of carboplatin ($P < 0.05$)

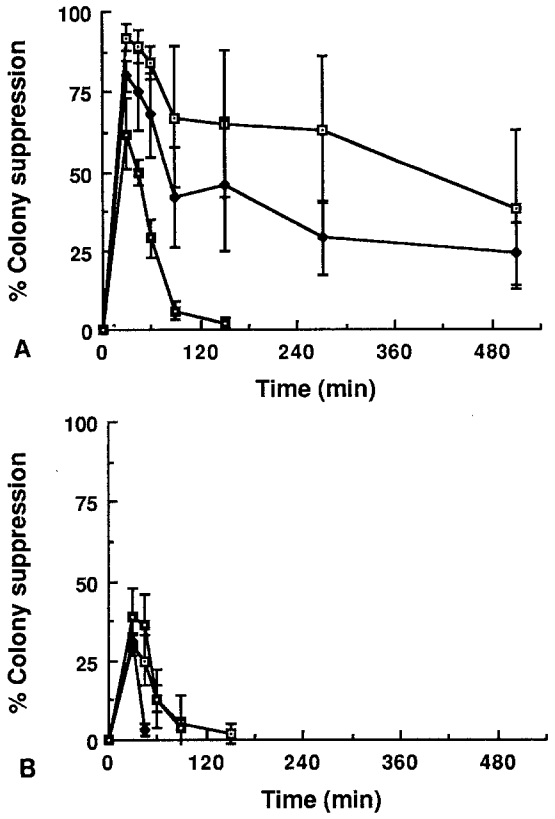

Fig. 1 A, B. Comparison of the antitumor activity of platinum compounds in 5 patients treated with 80 mg/m^2 cisplatin (■), 450 mg/m^2 carboplatin (◆), or 100 mg/m^2 254-S (-⊡-) as determined by bioassay. Human lung-cancer cell lines **A** NCI-H-69 and **B** PC-9 were used as target cells for the bioassay. The points represent the means ±SD of 5 patients

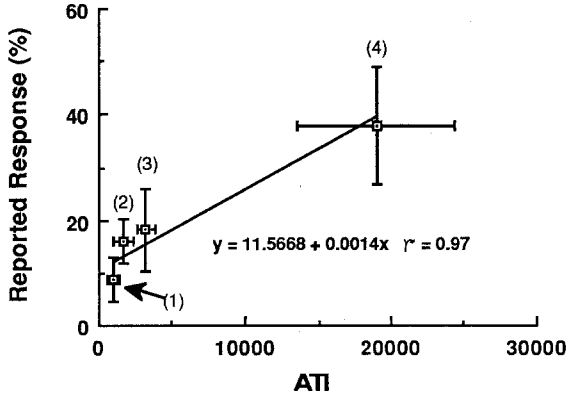

Fig. 2. The correlation between the ATIs shown in Table 3 and reported clinical response rates for cisplatin and carboplatin against SCLC and NSCLC (reviewed in Table 4). (*1*), carboplatin against NSCLC; (*2*), cisplatin against NSCLC; (*3*), cisplatin against SCLC; (*4*) carboplatin against SCLC. Each point represents the mean response rate and its 95% confidence interval and the mean ±SD for the ATI. The response rates for 254-S against SCLC and NSCLC are predicted to be 57%–67% and 16%–23%, respectively, according to the formula: [Reported Response (%)] = 11.5668+0.0014×[ATI]

Table 4. Phase II study of cisplatin and carboplatin in lung cancer patients

Investigators	Responders/ total patients	Response rate (%)	95% Confidence interval (%)
Cisplatin for NSCLC:			
Casper et al. [6]	1/18	5.5	5.5±10.5
Rossof et al. [25]	2/33	6	6 ± 8.1
Jager et ala. [17]	16/62	25.8	25.8±10.8
Fujita et al. [13]	6/42	14.2	14.2±10.5
Panettiere et al. [23]	19/118	16.1	16.1± 6.6
Total	44/273	16.1	16.1± 1.6
Cisplatin for SCLC:			
Levenson et al. [21]	1/18	5.5	5.5±10.5
Cavalli et al. [7]	4/11	36.3	36.3±28.4
Cavalli et al. [8]	5/23	21.7	21.7±16.8
Jager et al. [17]	4/13	30.7	30.7±25
Dombernowsky et al. [10]	3/28	10.7	10.7±11.4
Total	17/93	18.2	18.2±3
Carboplatin for NSCLC:			
Creekmore et al. [9]	3/27	11.1	11.1± 3.7
Kreisman et al. [18]	11/70	16	16 ± 3.1
Tamura et al. [38]	2/43	4.6	3.3± 5.9
Olver et al. [22]	0/41	0	<10
Total	16/181	8.8	4.7±12.9
Carboplatin for SCLC:			
Tamura et al. [37]	5/18	27.7	6.9±48.3
Smith et al. [34]	23/56	41	28.2±53.8
Total	28/74	37.8	26.8±48.8

Fig. 2, variation in the slope of the line may occur and the true relationship between the reported clinical responses and the ATI may not be linear, although the r value was 0.97.

From these retrospective analyses between ATIs for cisplatin and carboplatin and the clinical response, the clinical efficacy of 254-S against SCLC and NSCLC was prospectively predicted using Fig. 2. The response rates for 254-S against SCLC and NSCLC were predicted to be 40%–65% and 14%–16%, respectively, by this formula. 254-S was suspected of having almost same activity as cisplatin against NSCLC and of having the same, if not, activity than carboplatin against SCLC.

Discussion

It is difficult to predict the clinical antitumor activity of new agents before phase II and III trials have been conducted [19]. Even in the development of analogs, it is not known whether analogs will have superior antitumor activity against certain cancer cell types as compared with their parent compounds. There are some pitfalls to the prediction of the antitumor activity of new drugs because of the following reasons: (1) a difference in the in vitro antitumor activity between the daughter and the parent compound when they are compared at the same concentration; (2) a difference in pharmacokinetic behavior as well as peak

plasma concentration; and (3) a difference in the chemosensitivity of target tumors.

In the present study we demonstrated differences in the chemosensitivity of SCLC and NSCLC cell lines to platinum compounds. The clinical experiences suggest that SCLC is much more sensitive than NSCLC to most anticancer agents. Although our data was limited to cisplatin and its analogs, this clinical difference in the chemosensitivity of two types of lung cancer has also been demonstrated in cell lines of lung carcinoma. The same observation has been reported by Carmichael et al. [4]. Scheithauer et al. [30, 31] chose three human colorectal cancer cell lines and tested a variety of anticancer agents against these cell lines for the prediction of the antitumor activity of new agents using the Bactec assay. These authors evaluated the antitumor activity of a compound by using a ratio of the ID_{90}s of the three cell lines to 1/10 of the plasma concentrations clinically achievable in man. In their report, however, no information as to AUCs or pharmacokinetic behavior was considered in the evaluation of the drugs. Although we have no precise information about drugs other than platinum compounds, our data showed that both PC-9 and NCI-H-69 would be good target cell lines for the prediction of the antitumor activity to three platinum analogs against SCLC and NSCLC, respectively.

Pharmacokinetic analysis of the active free form of platinum revealed that the pharmacokinetic behavior of platinum given as 254-S was similar to that of platinum given as carboplatin, disappearing from plasma in a biphasic pattern. In contrast, free platinum from cisplatin disappeared monoexponentially. In the analysis of biological pharmacology carried out by measuring ex vivo drug pharmacodynamics using a bioassay, when PC-9 was selected as the target cell line, only the α-phase of the three platinum compounds was sufficient to decrease colony formation. In contrast, when the chemosensitive NCI-H-69 cell line was chosen as the target for bioassay, both the α- and β-phases of 254-S and carboplatin had high enough drug concentrations to kill these cells. This result suggests that the peak achievable concentration could be more important in the treatment of chemoresistant tumors such as NSCLC, whereas the pharmacokinetic behavior or AUC might also be important in the treatment of more chemosensitive lung cancer, as is the peak plasma concentration of anticancer agents.

The AUC is thought to be one of the most important pharmacokinetic parameters for the prediction of pharmacodynamic effects such as myelosuppression. Egorin et al. [11] indicated a promising strategy for the prediction of carboplatin-induced thrombocytopenia by measuring the AUC and the creatinine clearance. We have also demonstrated a similar approach in predicting platelet reduction by 254-S [29]; however, the AUCs for free platinum have less value for the comparison of the antitumor effects of different platinum compounds shown in this study, mainly due to differences in the antitumor activity of drugs, even when the platinum AUC values are the same. In addition, the AUC values for free platinum did not correlate with ATI/SCLCC or with ATI/NSCLC. This retrospective analysis showed good correlation between the clinical response rates reported for cisplatin and carboplatin against SCLC and NSCLC using ATIs as determined by bioassay, which means that the ex vivo pharmacodynamic parameter would be a good one for the comparison of the anticancer activity of different derivatives.

In vitro chemosensitivity testing is also a promising approach for the prediction of antitumor efficacy before clinical trial if we can use the pharmacokinetic behavior in man precisely as estimated by an animal model; however, our present understanding is that no reliable system has been established to predict the achievable plasma concentration or the pharmacokinetic behavior of investigational new anticancer drugs before their clinical use. In the present study we used plasma obtained from patients entered in a phase I clinical trial and applied the investigational platinum complex 254-S to detect the peak plasma concentration achievable at the standard dose of this agent, then analyzed the biological as well as the biochemical pharmacology. Comparison of the antitumor activity of three platinum compounds was achieved by determining the colony-suppressive effect on human lung-cancer cell lines (which was used as a common indicator) caused by drug-containing plasma from patients treated with these compounds. This approach is indeed effective for the prediction of the activity of analogs that inhibit cell growth by similar mechanism, but further investigations are necessary for the comparison of different kinds of anticancer agents. In addition, although the retrospective analysis demonstrated that the chemosensitivity of NCI-H-69 and PC-9 would have good predictive values for the clinical response of SCLC and NSCLC to cisplatin as well as carboplatin, further evaluation using other anticancer drugs should be performed.

A number of investigational drugs have been synthesized and applied in clinical use. Disease-oriented clinical phase II studies are necessary to determine the anticancer activity of new agents against certain types of tumors. However, ethical problems remain for the administration of new drugs to untreated cancer patients with treatable malignancies, such as SCLC, for which established standard chemotherapeutic regimens exist. Aisner [1] has proposed the possibility of new drug testing using a number of well-characterized SCLC cell lines. Our new approach creates another possibility for the prediction of the efficacy of investigational drugs. Based on the ATI values for 254-S, the response rate obtained for 254-S in SCLC and NSCLC could be predicted to be 40%–65% and 14%–16%, respectively, and suggested that 254-S would be as active against SCLC and NSCLC in this system. The phase II study of 254-S in lung cancer is now under way in Japan. Ariyoshi et al. [2] reported the preliminary results of a phase II study of 254-S in lung cancer; the response rates for 16 SCLC and 37 NSCLC patients were 44% and 19%, respectively. Our study also revealed a 15% response rate in 68 inoperable NSCLC patients (unpublished data); however, further studies are necessary to establish the validity of this new approach for predicting clinical response using other new anticancer agents.

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