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Antitumor Activity of Five New Platinum Complexes Having a Glycolate Leaving Ligand

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In an attempt to develop a new anticancer platinum complex with greater or equivalent antitumor activity but reduced side effects compared with cisplatin (CDDP), a series of new platinum complexes having a glycolate leaving ligand was synthesized. Among them, five complexes were selected for further development on the basis of adequate water solubility, low nephrotoxicity and high antitumor activity in a murine system. The chemosensitivity of these five complexes was examined in MTT assay against two human pulmonary adenocarcinoma cell lines, PC-9 and PC-14, and two human stomach adenocarcinoma cell lines, MKN-45 and KATO III. Their IC_{50} and relative antitumor activity (RAA) values were compared with those of CDDP and 254-S, a second-generation platinum complex with a glycolate leaving ligand under phase III clinical trial. The lowest mean IC_{50} value was observed in CDDP, followed by SKI 2034R and SKI 2033R. In this study, the antitumor activity was evaluated in terms of RAA values and SKI 2034R showed the highest RAA value. The order of RAA values was SKI 2034R > CDDP > SKI 2032R > SKI 2033R > SKI 2030R > SKI 2029R > 254-S. Based on the RAA order, we have recommended SKI 2034R as the most promising candidate for further development of a clinically useful platinum complex.

Key Words : *Platinum complexes, Glycolate, Antitumor activity, Lung, Stomach, Cancer cell line*

INTRODUCTION

Since the introduction of cis-dichlorodiammineplatinum(II) (cisplatin, CDDP) into clinical practice, CDDP has been one of the major components of combination regimens for many kinds of human solid cancers, because of its potent and broad spectrum antitumor activity (Rosenberg et al., 1969 ; Loehrer and Einhorn, 1984). However, the strong side effects of CDDP, such as nephrotoxicity, gastrointestinal toxicity and neurotoxicity, have been serious problems which affect therapeutic results by restricting further administration of CDDP and the quality of life during the remaining period of life (Von Hoff et al., 1979 ; Pinzani et al., 1994). Because of these side effects, a number of platinum analogues have been synthesized to develop alternative complexes having greater or

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equivalent antitumor activity but carrying less severe side effects compared with CDDP (Brown et al., 1982 ; Hydes and Russell, 1988 ; Kim et al., 1994 ; Kim et al., 1995).

Recently we have synthesized a new series of cis-glycolato[(4R, 5R)-4,5-bis(aminomethyl)-2-isopropyl-1,3-dioxolane] platinum(II) complexes (Kim et al., 1994). Among them, we have selected five complexes for further development on the basis of their physicochemical properties, such as solubility and stability, and the high antitumor activity and low nephrotoxicity in in vitro and in vivo murine system. In an attempt to develop a more effective anticancer platinum complex for clinical use, we have examined the antitumor activities of five complexes against human cancer cell lines and compared their activities with those of CDDP and glycolato-O, O'-diammineplatinum(II) (254-S), because 254-S is the only platinum complex known to have a glycolate leaving ligand in clinical development (Majima H, 1991 ; Fukuda et al., 1994). The results of this study suggest that SKI 2034R is the most promising candidate for further development as a clinically useful anticancer drug.

MATERIALS AND METHODS

Cells

Two human stomach adenocarcinoma cell lines, MKN-45 and KATO III, and two human pulmonary adenocarcinoma cell lines, PC-9 and PC-14, were used in these experiments. These cell lines were kindly supplied by Dr. N. Saijo, National Cancer Center Hospital, Tokyo, Japan. All cell lines were grown in RPMI-1640 medium (Gibco Laboratory, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco), penicillin (100 U/ml) and streptomycin (100 μg/ml) (RPMI-FBS). Cells were incubated at 37℃ in a humidified atmosphere of 5% CO_2 and subcultured once or twice per week as necessitated by the growth rate.

Drugs

CDDP was obtained from the Sigma Chemical Co. (St. Louis, MO, USA). 254-S, SKI 2029R (NSC D643686-R), SKI 2030R (NSC D643688-T), SKI 2032R (NSC D642488-P), SKI 2033R (NSC D643687-S) and SKI 2034R (NSC D642489-Q) were synthesized at the Life Science Research Center, Sunkyong Industries, Korea, as previously described (Totani et al., 1986 ; Kim et al., 1994). The chemical structures of these platinum complexes are shown in Fig. 1. The stock solutions of these complexes, CDDP and 254-S were made by diluting with distilled water and stored at -70℃. Immediately before each experiment, these stock solutions were dissolved in RPMI-FBS to the required concentrations.

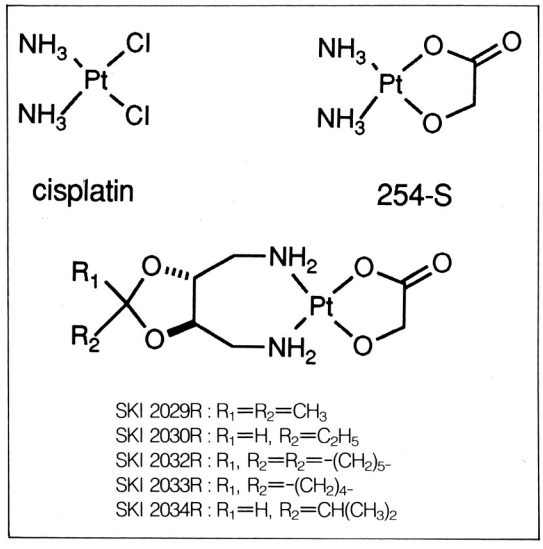

Fig. 1. Chemical structures of platinum complexes.

Chemosensitivity

The MTT assay was performed as previously described (Carmichael et al., 1983). In brief, single cell suspensions were prepared by mechanical disaggregation and/or trypsinization. After viability of cells was confirmed over 95% by trypan blue dye exclusion test, 135 μl of cell suspensions were plated into each well. The number of cells plated and incubation time were determined in the standard and growth curves of each cell line. The final concentrations of the cells (/well) of PC-9, PC-14, MKN-45 and KATO III were 2×10^3, 3×10^3, 5×10^3 and 5×10^3, respectively. Drugs were added to each well in 15 μl volume at various concentrations. After the plates were incubated at 37℃ in a humidified incubator with 5% CO_2 for 4 days, 15 μl of 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT, Sigma) dissolved in phosphate-buffered saline (5 mg/ml) were added to each well. The

Table 1. Solubility, molecular weight, LD_{50} and peak plasma concentration (PPC) of six glycolate derivatives of platinum complexes.

Platinum complexes	Solubility (mg/ml)	Molecular weight (dalton)	LD_{50} (mg/kg)	PPC (μg/ml)
SKI 2029R	17.5	429.5	34.0	2.34
SKI 2030R	>50	429.3	38.5	2.57
SKI 2032R	2.9	469.4	60.2	3.59
SKI 2033R	5.8	455.4	43.5	2.81
SKI 2034R	>30	443.4	60.0	3.59
254-S	14.0	303.2	22.2	1.69

1) Solubility, molecular weight and LD_{50} of platinum complexes in ICR mice were obtained from the Life Science Research Center, Sunkyong Industries, Korea.
2) Water solubility was determined at 20℃ by UV scanning at 220 nm.

plates were incubated again for 4 hr. After the plates were centrifuged at 450g for 5 min, the supernatant was aspirated and 150 μl of dimethyl sulfoxide (Sigma) were added to each well. The plates were then agitated on a plate shaker for 10 min to solubilize formazan crystals and the optical density (OD) was read at 540 nm on Titertek Multiskan Plus MKII (Flow Laboratories, Finland). Each experiment was performed in triplicate and repeated three times.

Evaluation of antitumor activity

The IC_{50} value (μg/ml) was defined as the concentration of drug producing 50% inhibition of growth (OD) and determined graphically from the dose-response curve of each cancer cell line. The relative antitumor activity (RAA) was defined as the ratio of peak plasma concentration (PPC)(μg/ml) to IC_{50} value and used to compare the antitumor activity (Takahashi et al., 1987 ; Horiuchi et al., 1988 ; Ohe et al., 1989 ; Hong et al., 1995). The PPC for CDDP was considered to be 2.49 μg/ml which was reported in the previous phase I study (Albert and Georgechen, 1980). On the other hand, the accurate pharmacokinetic data of SKI 2029R, SKI 2030R, SKI 2032R, SKI 2033R and SKI 2034R in humans have not been published. Therefore, the predictive PPC values for these platinum complexes were calculated by the following equation : log(predictive PPC)=0.755×log (mice LD_{50})-0.788 (Scheithauer et al., 1986). The LD_{50} of the new complexes and predictive PPC values are listed in Table 1. Statistical difference was determined by analysis of variance (ANOVA) with Duncan's multiple-range test and Tukey's studentized range test. A p value of <0.05 was considered to be statistically significant.

RESULTS

All five platinum complexes, CDDP and 254-S inhibited the survival of cancer cells dose-dependently (Fig. 2)(data not shown except for MKN-45). The IC_{50} values of the cancer cells are listed in Table 2. CDDP showed the lowest mean IC_{50} value of 0.73 ± 0.29 μg/ml, while the order of IC_{50} value was CDDP < SKI 2034R < SKI 2033R < SKI 2032R < SKI 2030R < 254-S < SKI 2029R. In this study, the antitumor activity of complexes was compared in terms of the RAA values and the order of antitumor

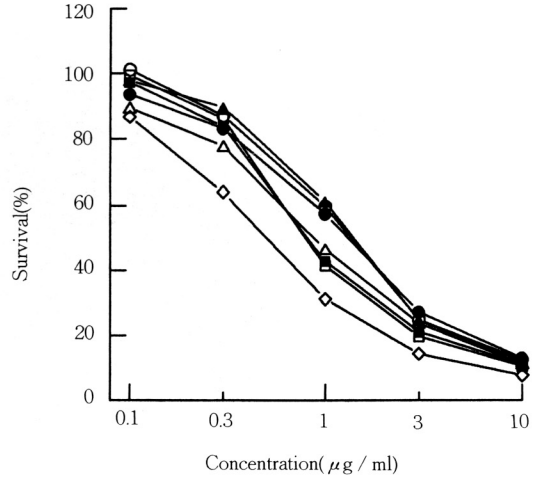

Fig. 2. Dose-response curves of seven platinum complexes : SKI 2029R(●), SKI 2030R (○), SKI 2032R (▲), SKI 2033R (△), SKI 2034R (■), 254-S (□) and cisplatin (◇) on a human stomach adenocarcinoma cell line, MKN-45. Each point represents the mean of three experiments.

Table 2. IC_{50} values for five new platinum complexes, cisplatin and 254-S in human pulmonary adenocarcinoma cell lines, PC-9 and PC-14 and human stomach adenocarcinoma cell lines, MKN-45 and KATO III.

	PC-9	PC-14	NKN-45	KATO III	Total
SKI 2029R	2.70 ± 0.65	0.82 ± 0.46	1.32 ± 0.35	3.00 ± 0.40	1.96 ± 0.91
SKI 2030R	2.30 ± 0.41	0.59 ± 0.36	1.31 ± 0.37	1.80 ± 0.10	1.50 ± 0.63
SKI 2032R	2.05 ± 0.25	0.89 ± 0.46	0.98 ± 0.44	1.06 ± 0.44	1.24 ± 0.47
SKI 2033R	1.93 ± 0.30	0.62 ± 0.18	0.81 ± 0.13	1.08 ± 0.13	1.11 ± 0.50
SKI 2034R	1.98 ± 0.27	0.54 ± 0.22	0.78 ± 0.03	0.94 ± 0.16	1.06 ± 0.55
254-S	1.74 ± 0.40	0.73 ± 0.35	1.30 ± 0.43	3.00 ± 0.90	1.69 ± 0.83
CDDP	1.07 ± 0.46	0.41 ± 0.11	0.47 ± 0.15	0.94 ± 0.30	0.73 ± 0.29

Values represent the mean(μg/ml) ± SD of three experiments.

activity was considered to be in accordance with the order of RAA. As shown in Table 3, the highest RAA value was observed in SKI 2034R, with the order to be SKI 2034R > CDDP > SKI 2032R > SKI 2033R > SKI-2030R > SKI 2029R > 254-S. On the other hand, the complexes with significant differences from the RAA value of 254-S were CDDP and SKI 2034R ($p < 0.05$).

DISCUSSION

One of the major problems clinically encountered with the use of CDDP is the serious side effects (Von Hoff et al., 1979; Pinzani et al., 1994). The other problems are the lack of antitumor activity for certain kinds of cancers, emergence of acquired drug resistance and low solubility in water, etc. (Hong et al., 1988; de Graeff A et al., 1988). To overcome these drawbacks of CDDP, numerous platinum analogues have been synthesized and evaluated for their antitumor activity and toxicity, because the search for analogues is one of the most efficient methods to develop a new active drug.

Most platinum complexes consist of platinum atom, carrier ligand and leaving ligand. A number of previous preclinical and clinical investigations have provided evidence that there is a considerable relationship between chemical structures of platinum complexes and biological activity: Carrier ligand is closely related to the antitumor activity and spectrum, while leaving ligand affects the stability, solubility and toxicity (Connors et al., 1979; Kim et al., 1994).

Recently we have synthesized a series of new platinum complexes having a glycolate as a leaving ligand but different carrier ligand and selected five complexes for further evaluation. These complexes were selected for the high solubility and stability in aqueous solution and the high antitumor activity and low nephrotoxicity in a murine system. 254-S, one of the second-generation platinum complexes with a glycolate leaving ligand, is currently undergoing phase III study in Japan. 254-S was reported to have equivalent or superior activity to CDDP against various murine and human cancers, but much less nephrotoxicity (Shiratori et al., 1985; Majima H, 1991).

The antitumor activity of drugs should be examined and interpreted to reflect their clinical effectiveness. The effectiveness of drugs is mainly affected by the sensitivity of cancer cells to the drug and the concentration of the drug achieved after drug administration.

Table 3. Relative antitumor activity (RAA) values for five new platinum complexes, cisplatin and 254-S in human pulmonary adenocarcinoma cell lines, PC-9 and PC-14 and human stomach adenocarcinoma cell lines, MKN-45 and KATO III.

	PC-9	PC-14	MKN-45	KATO III	Total
SKI 2029R	0.87	2.85	1.77	0.78	1.57 ± 0.84*
SKI 2030R	1.12	4.36	1.96	1.43	2.22 ± 1.27
SKI 2032R	1.75	4.03	3.66	3.39	3.21 ± 0.87
SKI 2033R	1.46	4.53	3.47	2.60	3.02 ± 1.13
SKI 2034R	1.81	6.65	4.60	3.82	4.22 ± 1.73+
254-S	0.97	2.32	1.30	0.56	1.29 ± 0.65
CDDP	2.33	6.03	5.30	2.65	4.08 ± 1.61+

*, mean ± SD

+, $p<0.05$, different from RAA value of 254-S

From this point of view, IC_{50} value and cell survival rate at a certain concentration are not good parameters to predict clinical effectiveness, because the optimal dose and pharmacokinetic characteristics, such as plasma concentration of the drug, may be different between drugs. On the other hand, the RAA values have been used to evaluate clinical effectiveness in humans before the clinical trials, because they are determined by the two main parameters mentioned above, sensitivity to the drug and plasma concentration of the drug (Takahashi et al., 1987; Horiuchi et al., 1988; Ohe et al., 1989; Hong et al., 1995). The antitumor activities of platinum complexes, in this study, were compared with those of CDDP and 254-S using RAAs.

The PPC of CDDP was considered to be 2.49 μg/ml, which was reported from the previous phase I study (Albert and Georgechen, 1980). However, the PPCs of the new platinum complexes are not available by the termination of phase I and pharmacokinetic study. Therefore, in the case of new complexes, a calculated PPC has been widely used. Although there may be some discrepancies between the calculated PPC and actual PPC due to the difference in the pharmacokinetic behaviors of the drugs, the antitumor activity, in this study, was compared in terms of RAAs which were calculated with predictive PPCs.

In this study, we have compared the antitumor activity of five new platinum complexes with that of 254-S in terms of RAA, demonstrating that all five complexes were superior to 254-S and that SKI 2034R showed the highest RAA value. SKI 2034R is a new synthetic platinum complex which has 2-isopropyl-1,3-dioxolane-4,5 bis(aminomethane) as a carrier ligand and glycolate as a leaving ligand. After due consideration of the order of RAA obtained from the present study, we came to the conclusion that SKI 2034R may be the most promising candidate for the development of a new anticancer drug. Since many platinum complex candidates have been excluded due to unexpected serious side effects in early clinical trials, careful further evaluation, both in vitro and in vivo animal tumor systems, should be undertaken to predict the clinical activity and toxicity of this complex, SKI 2034R, before clinical trials.

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