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Selective Cytotoxicity of Novel Platinum(II) Coordination Complexes on Human Bladder Cancer Cell-Lines and Normal Kidney Cells

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Cisplatin is often effective in cancer treatment, but its clinical use is limited because of its nephrotoxicity. We have synthesized new platinum(II) coordination complexes (PC-1 & PC-2) containing trans-*l* and cis-1,2-diaminocyclohexane (DACH) as carrier ligands and L-3 -phenyllactic acid (PLA) as a leaving group with the aim of reducing nephrotoxicity but maintaining its anticancer activity. In this study, new platinum(II) complex compounds were evaluated for selective cytotoxicity on cancer cell-lines and normal kidney cells. The new platinum complexes have demonstrated high efficacy in the cytotoxicity against human bladder carcinoma cell-lines (T-24/HT-1376). The cytotoxicity of these compounds against rabbit proximal renal tubular cells and human renal cortical tissues, was determined by MTT assay, the [3H]-thymidine uptake and glucose consumption test, and found to be quite less than those of cisplatin. Based on our results, these novel platinum compounds appear to be valuable lead compounds with high efficacy and low nephrotoxicity.

Key Words: Selective cytotoxicity, Platinum(II) complex, Human bladder carcinoma cells, Normal kidney cells

INTRODUCTION

The platinum coordination complexes were first identified by Rosenberg and coworkers as cytotoxic agents in 1965. The observed that a current delivered between platinum electrodes produced inhibition of *E. coli* proliferation. The inhibitory effects on bacterial replication were later ascribed to the formation of inorganic platinum-containing compounds in the presence of ammonium and chloride ions (Rosenberg et al, 1965, 1967).

The introduction of the square-planar complex cis-diaminodichloroplatinum (II) (Cisplatin; CDDP) into the clinical treatment of cancer has resulted in excellent response rates for some tumor type, especially testicular and ovarian cancer (Rosenberg et al, 1969).

CDDP was the most active of these substances in experimental tumor systems and has proven to be of great clinical value (Rosenberg et al, 1973). CDDP has broad activity as an antineoplastic agent, and is especially useful in the treatment of epithelial malignancies. It has become the foundation for curative regimens for advanced testicular cancer and has notable activity ovarian cancer and cancer of the head and neck, bladder, esophagus, and lung. While the unfavorable nephrotoxicity profile of CDDP has been overcome by the development of the second-generation agent, carboplatin, there remains an unquestionable need for additional platinum(II)coordination complexes which have more favorable therapeutic indices and circumvent resistance (Calvert et al, 1985). As with other cancer chemotherapeutic agent, cellular resistance to the clinically used platinum agent, CDDP and carboplatin, represents a major clinical limitation to the efficacy (Mangioni et al, 1989; Einhorn, 1990). The nephrotoxicity that accompanies during the administration of CDDP has been investigated in considerable details (Ward & Fauvie, 1976; Groth et al, 1986). It is known that cisplatin undergoes hydrolysis to only a modest extent in the serum because the chloride concentration is high in the serum. But the hydrolysis of CDDP occurs to a much lower in the intracellular space (Lim & Martin, 1976; Litterest, 1981). The species formed from CDDP by hydrolysis are known to be more reactive and more nephrotoxic than the parent compound, CDDP (Rosenberg, 1978; Daley-Yates & McBrien, 1982, 1984, 1985), but quantitative information on their relative nephrotoxicity is limited.

Consequently, there is much interest in obtaining agents that have less toxicity and more favorable therapeutic indices. To accomplish this goal, we synthesized a new platinum analog containing trans-*l* and cis-1,2-diaminocyclohexane (DACH) as carrier ligands and L-3-phenyllactic acid (PLA) as a leaving group. Previously, we have shown that this new platinum complex containing DACH has much less nephrotoxicity than CDDP (Jung et al, 1998).

The antitumor activity of platinum complexes containing DACH as a carrier ligand were investigated by Connors et

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ABBREVIATIONS: CDDP, cisplatin; DACH, diaminocyclohexane; PLA, phenyllactic acid; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

al (1972) and Gale et al (1974). Kidani (1985) synthesized oxaliplatin using oxalic acid with selected trans-*l*-DACH among trans-*d* and cis-isomers.

In the present study, we evaluated the *in vitro* antitumor activities of the new platinum complexes on the human bladder cancer cell-lines (T-24 and HT-1376) and the cytotoxicity on rabbit proximal tubular cells, human renal cortical cells and histocultured human renal cortical tissues.

METHODS

Materials

Dulbecco's Modified Eagle's (DMEM), Ham's Nutrient Mixture F12 (F12) and RPMI-1640 media were purchased from GIBCO (Grand Island, NY). Cisplatin, hormones, transferrin, and other chemical were purchased from Sigma Chemical Co. (St. Louis, MO). Powdered medium, EDTA-trypsin inhibitor were obtained from Life Technologies (Grand Island, NY). Class IV collagenase was obtained from Worthington Co. (Freedhold, NY). Iron oxide was prepared by the method described by Cook and Pickering (1958). Stock solutions of iron oxide in 0.9% NaCl were sterilized in an autoclave and diluted with phosphate-buffered saline (PBS) prior to the application. The novel platinum coordination complexes, Pt(II)(trans-*l*-DACH)(PLA)[PC-1] and Pt(II)(cis-DACH)(PLA)[PC-2]; (Fig. 1) the PCs were synthesized in the Department of Pharmacochemistry, Kyung Hee University College of Pharmacy. This agent was dissolved in sterilized cell culture media prior to usage.

Cell cultures

Kidney cell cultures were maintained in a humidified, 5% CO_2 incubator at 37°C. The basal culture medium, 50 : 50 mixture of Dulbecco's modified Eagle's medium and Ham's F12 (DME/F12) medium, was supplemented with 15 mM HEPES buffer, 1.2 mg/ml sodium bicarbonate, 92 IU/ml penicillin, and 200 μg/ml streptomycin. Primary rabbit kidney proximal tubular cells were cultured in serum-free basal medium supplemented with bovine insulin (5 μg/ml), human transferrin (5 μg/ml), and hydrocortisone (5×10^{-8} M). Growth supplements (bovine insulin, human transferrin and hydrocortisone) were immediately added to the serum free basal medium before their use for tissue culture.

Primary rabbit kidney proximal tubular cells: Primary culture were initiated from purified rabbit kidney tubules by a modification of the method described by Chung et al (1982). Each growth study was conducted with an individual primary culture set derived from purified proximal tubules obtained from a single rabbit kidney. Individual kidneys were obtained from male New Zealand White rabbits (2~2.5 kg). The kidney of a male New Zealand White rabbit were perfused via the renal artery, first PBS, and subsequently with DME/F12 containing 0.5% iron oxide (wt/vol) until the kidneys turned gray-black in color. Renal cortical slices were homogenized with 4 strokes of a sterile Dounce homogenizer (type A pestle, Bellco, Vineland, NJ) and the homogenate was poured first through a 263-and then a 83-mesh filter. Tubules and glomeruli on top of the 83-mesh filter were transferred into sterile serum-free modified DME/F12 medium containing a magnetic stir bar. Glomeruli containing iron oxide were removed the magnetic stir bar. The remaining purified proximal tubules were briefly incubated in serum-free modified DME/F12 containing the 3 supplements (bovine insulin, human transferrin, hydrocortisone) and transferred to culture dishes. The culture were maintained in a humidified 5%CO_2/95% air environment at 37°C. The purified rabbit kidney proximal tubules attached to the culture dish within a day of plating. The medium was replaced the day after plating so as removed unattached tubules. The medium was replaced every two days thereafter. During the initial 4 days, cell outgrowth occurred from the attached tubules in culture.

Primary human kidney cortical cells: Normal human kidney tissues were obtained at the time of radical nephrectomy in patients with renal cell carcinoma. Human kidney cortical tissues were washed 3 or 4 times with DME/F12 medium (1 : 1 ratio) supplemented with penicillin G/streptomycin. A single-cell suspension was obtained by mechanical disaggregation with a sterilized

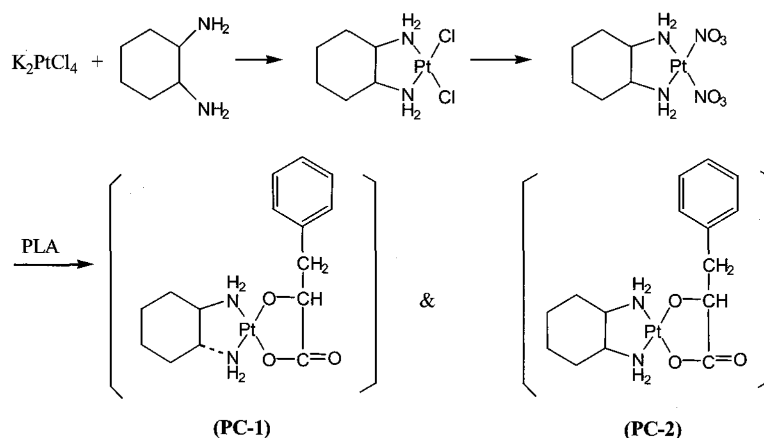

Fig. 1. Synthetic process of Pt(II) complexes containing 1,2-diaminocyclohexane isomer and L-3-phenyllactic acid. DACH: trans-*l*, cis-1,2-diaminocyclohexane, PLA: L-3-phenyllactic acid, PC-1: Pt(II)(trans-*l*-DACH)(PLA), PC-2: Pt(II)(cis-DACH)(PLA).

surgical knife and subsequent incubation with collagenase (0.124 mg/ml) and trypsin inhibitor (2.5 mg/ml) for 2 minutes. The process was stopped by centrifugation (1000 rpm for 5 minutes) and the particles of the kidney cortical tissues were suspended in DME/F12 medium supplemented with insulin (5 μ g/ml), transferrin (5 μ g/ml), hydrocortisone (5×10^{-8} M), triiodothyronine (5 μ g/ml), prostaglandin E1 (5×10^{-8} M) and 1% fetal bovine serum (FBS) (GIBCO, Grand Island, NY). These suspended cells were seeded in a culture dish and kept in an incubator at 37°C with a highly humidified 5% CO_2/95% air. After 2 weeks of incubation, the cells were confluent and used for experimental (Jung et al, 1998).

Culture of cancer cell lines: Monolayer cultures of cancer cell lines were grown with HT-1376 and T-24 (human bladder carcinoma cells). These cell-lines were cultured in RPMI 1640 medium supplemented with 10% FBS, 20 mM sodium bicarbonate, 15mM HEPES, 92 units/ml penicillin G and 200 mcg/ml streptomycin. All the cells were incubated in humidified 5% CO_2/95% air at 37°C.

In vitro antitumor activity

Cancer cell lines were cultured in the growth media for each line in an incubator with a highly humidified 5% CO_2/95% air atmosphere at 37°C. After 3 days of incubation, all cell lines were dissociated with 0.025% trypsin-EDTA for dispersal and centrifuged at 1,000 rpm for 5 minutes. The pellets were suspended with fresh medium. Individual wells of a 96-well tissue culture microtiter plate were inoculated with 0.1ml of the appropriate medium containing 10^5 cells. New platinum(II) coordination complex and cisplatin were added in various concentration. After 48 hours of incubation, 0.05 ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/ml) was added to each well. After 4 hours of exposure, 0.05 ml of DMSO was added to each well and absorption rates at 630nm were measured using an ELISA reader (Shimoyama et al, 1989).

Cytotoxicity on normal kidney cells

The confluent primary rabbit kidney proximal tubular and human kidney cortical cells were disaggregated by using 0.02% EDTA in 0.05% trypsin. Single-cell suspension was then produced by centrifugation (1,000 rpm, 10 minutes) and resuspension in DME/F12 medium. This suspension was seeded at a cell density of 10^5 cells per well on a 96-well plate, with 100 μl of medium per well. Drugs were added in various concentrations (final concentration: 5, 25, 50, 250 and 500 μM) and the cell cultures were incubated for 48 hours in an incubator a highly humidified atmosphere of 5% CO_2/95% air at 37°C. Thereafter, 50 μl of the medium containing MTT (5 mg/ml) was removed and the wells were washed with PBS, and then 50 μl of DMSO was added to each well to solubilize the precipitates. Then the plates were transferred to an ELISA reader to measure the absorbance of the extracted dye at 630nm. All experiments were performed at 3 times, with 6 wells for each concentration of new platinum(II) coordination complexes and cisplatin (Mossman, 1973).

Thymidine uptake

Primary cultured proximal tubular and cortical cells were seeded in each well of a 24-well plate at a cell density of 10^5 cells per well. After 1hour of incubation, platinum (II) coordination complexes were added, and the cells, were incubated for 48 hours in a humidified incubator containing an atmosphere of 5% CO_2/95% air at 37°C. Thereafter, [^{3}H]-thymidine (1 μCi/ml specific radioactivity) was added to each well, and cells were incubated for 24 hours in the same humidified incubator.

After trypsin-EDTA treatment, all cells were collected and washed twice with 10% TCA and phosphate buffer. The cells were then solubilized with 0.5M NaOH for 2 hours at 37°C. The cells were neutralized with 0.5 M HCl and a scintillating cocktail was added (Scint-AXF, Packard, CT). Then the amount of radioactivity uptake by the cells was determined in a β-counter (Beckman, LS 5000TD).

Histoculture of human renal cortical tissue

Human renal cortical tissues, identified by frozen section at the time of radical nephrectomy in patients with renal cell carcinoma, were transported in a sterile container in the laboratory located near the operating room. Human renal cortical tissues were divided into 2 to 3 mm diameter pieces and five pieces were placed on top of previously hydrated Spongostan gel ($1 \times 1 \times 1$ cm) (Health Design Indust. Rochester, NY). Each gel occupied one well of a six-well plate. Three milliliter of Eagle's Minimal Essential Medium (MEM) (GIBCO Grand Island, NY) supplemented with 10% fetal bovine serum (GIBCO), 50 μg/ml gentamicin final concentration, and cefotaxime (Hoechst, Somerville, NY) at a final concentration of 1 of 1 μg/ml were added. The final volume of medium was sufficient to reach the upper gel surface without immersing it. Covered culture plates were maintained in a humidified, 5%CO_2 incubator at 37°C. The cultures underwent sterile media changes every 72 hours. Histocultured was continued up to 3 weeks after explanation. Histocultured specimens were incubated in medium with 10^{-4} M of the experimental new platinum analogs or CDDP for 72 hours. The drug treatment was performed at 3 periods after tissue explanation. One period constituted 3 days. After drug treatment, the specimens were washed with PBS and fresh medium (Freeman & Hoffman, 1986; Chang et al, 1992).

Glucose consumption from histocultured specimens

This study was performed essentially as described by Chang et al (1993). In briefly, 50 μl culture medium were removed every 24 hours for determination of medium glucose content in triplicate using the HK 20 assay kit from Sigma (St Louis, MO). Measurements were made by monitoring the change in optical density at 340 nm due to the reduction of NAD catalyzed by hexokinase with the glucose substrate before and after treatment. The glucose content of the medium plotted in semilog form vs time using the Sigma plot program (Systat. Inc. Evanston, IL). The half-life of glucose was calculated from the slope parameter of this model using the equation $t1/2=0.693/s$ where s=slope of the best fit linear regression line natural log of the glucose concentration plotted vs time. The glucose content of the medium was measured daily for 3 day. The log values over 3 days were plotted vs time and the slope of the best-fit line was taken as the glucose consumption rate during the 3-day measurement period (one period).

RESULTS

In vitro antitumor activity

Efficacy of the new platinum complexes on cancer cell lines in monolayer culture: Human bladder cancer cell lines were treated with various concentrations of the new platinum complexes (PC-1 and PC-2) and CDDP. The concentrations were determined by previously reported CDDP effects on various cancer cell lines (Jung et al, 1999). The cytotoxic concentrations (CC_{50}) of PC-1 on HT-1376 and T-24 were 25.8±4.36 and 23.5±3.79 (μM), and of PC-2 were 26.4±4.18 and 25.3±4.15 (μM), respectively. These findings were similar to CDDP (27.2±4.36 or 25.0±3.54 (μM). Antitumor activites of these platinum(II) coordination complexes against human bladder carcinoma cells exhibit concentration-dependent increments and quite comparable to that of CDDP (Table 1).

Nephrotoxicity

Selective cytotoxicity: To investigate nephrotoxicity, primary cultured rabbit and human normal kidney cells were incubated with various concentrations of CDDP and the new compounds for 48 h. The cytotoxicity of the new compounds (PC-1 and PC-2) on primary cultured normal kidney cells were lower, by about 9 or 10 times, than that of CDDP (Table 2, 3).

Effect on [^{3}H]-thymidine uptake by normal kidney cells: [^{3}H]-thymidine uptake was measured from primary cultured rabbit proximal tubular cells and renal cortical cells of human kidney. These cultured cells were treated with 5×10^{-5} M of CDDP, PC-1 or PC-2 and showed a thymidine uptake of 8.4, 56.3 and 59.3% (rabbit kidney) and 8.2, 55.0 and 58.8% (human kidney), respectively. These results indicate that the cytotoxicity of the new platinum complex compounds was significantly less than that of CDDP (Table 4, 5).

Glucose consumption: The glucose consumption rate was calculated as the medium glucose-content half-life. The glucose half-life on the control was almost steady and the average was 60.14 hours. However, the medium glucose half-life varied with the different drugs tested, depending on the drug concentrations of CDDP and experimental new platinum analogs (PC-1 and PC-2). In measuring the glucose consumption, one period was defined as more 3 measurements per day in a 4-week culturing period of human renal cortical tissue. The glucose half-life before adding PC-1 and PC-2 (10^{-4} M) was approximately 57~62 hours and did not show any significant increase for third periods, and then increased to over 75 hours from the 4th period up to the 7th period. However, the effects of PC-1 and PC-2 were less marked than that of CDDP. The half-lifes of the medium glucose content were longer in

Table 1. *In vitro* cytotoxicities of new platinum(II) complexes (PC-1 and PC-2) and cisplatin(CDDP) on the human bladder carcinoma cell-lines

Compounds	CC_{50} (μM)	
	HT-1376	T-24
CDDP	27.2±4.36	25.0±3.54
PC-1	25.8±3.75	23.5±3.79
PC-2	26.4±4.18	25.3±4.15

CC_{50} indicates mean cytotoxic concentration with MTT assay; each value is the mean±S.E. of five experiments; PC-1, [Pt(II)(trans-*l*-DACH) (PLA)]; PC-2, [Pt(II)(cis-DACH)(PLA)] DACH, 1,2-diaminocyclohexane; PLA, L-3-phenyllactic acid.

Table 2. Cytotoxic activity of new platinum(II) complexes (PC-1 and PC-2) and cisplatin (CDDP) on the primary cultured proximal-tubular cells of rabbit kidney

Compounds	CC_{50} (μM)[a]	P/C[b]	P-values
CDDP	26.8±4.05	–	–
PC-1	234.2±33.14	8.74	<0.01
PC-2	242.5±35.06	9.05	<0,01

[a]Mean cytotoxic concentration by the MTT assays. [b]The ratio is CC_{50} value of the new Pt(II) complexes divided by CC_{50} value of cisplatin. [c]Each value is the mean±SE of five experiments.

Table 3. Cytotoxic activity of new platinum(II) complexes (PC-1 and PC-2)and cisplatin (CDDP) on the primary cultured cortical cells of human kidney

Compounds	CC_{50} (μM)[a]	P/C[b]	P-values
CDDP	24.7±3.75	–	–
PC-1	243.5±41.24	9.86	<0.01
PC-2	248.6±38.53	10.07	<0.01

[a]Mean cytotoxic concentration by the MTT assays. [b]The ratio is CC_{50} value of the new Pt(II) complexes divided by CC_{50} value of cisplatin. [c]Each value is the mean±SE of five experiments.

Table 4. Effect of platinum (II) coordination complexes (PC-1 and PC-2) *and cisplatin* (CDDP) *on* ^{3}*H-thymidine incorporation into* primary cultured proximal tubular cells of rabbit kidney

Compounds	^{3}H-thymidine uptake (cpm/10^5 cells)	Uptake rate (%)
Control	575.8±70.3	100.0
CDDP	48.1±6.5	8.4
PC-1	324.0±36.9	56.3
PC-2	341.5±43.2	59.3

Concentration of platinum(II) coordination complexes in culture medium: 5×10^{-5} M. Values are mean±S.E, All the incorporations were determined in triplicate. PC-1; [Pt (II)(*trans-l*-DACH)], PC-2; [Pt(II)(*cis*-DACH)(PLA)].

Table 5. Effect of platinum(II) coordination complexes (PC-1 and PC-2) and cisplatin (CDDP) on ^{3}H-thymidine incorporation into primary cultured cortical cells of human kidney

Compounds	^{3}H-thymidine uptake (cpm/10^5 cells)	Uptake rate (%)
Control	592.4±65.3	100.0
CDDP	48.5±7.7	8.2
PC-1	325.9±41.5	55.0
PC-2	348.6±44.2	58.8

Concentration of platinum(II) coordination complexes in culture medium: 5×10^{-5} M. Values are mean±S.E, All the incorporations were determined in triplicate. PC-1; [Pt(II)(*trans-l*-DACH)(PLA)], PC-2; [Pt(II)(*cis*-DACH)(PLA)].

Table 6. Change of glucose half-life on histocultured human renal cortical tissue specimens that were with 10^{-4} M cisplatin and experimental drugs (PC-1 and PC-2) for 72 hours exposure

Compounds	Glucose Half-life (Hours, M±S.E)						
	1	2	3	4	5	6	7 (periods)
CDDP	56.8±7.14	59.1±8.45	60.6±9.07	322.5±41.8	358.0±44.3	331.6±45.1	292.5±31.9
PC-1	58.5±8.27	60.9±7.55	61.3±8.42	64.1±9.16	65.7±9.05	70.5±9.45	74.8±9.43
PC-2	60.4±7.70	61.2±8.08	62.5±8.06	65.4±8.71	68.0±8.77	71.4±8.80	75.5±9.18

One period constitutes 3 days. All the experimental histocultures had their own control that was pretreatment three period. Glucose half-life on control specimen was very steady. CDDP; Cisplatin, PC-1;[Pt (*trans-l*-DACH) (PLA)], PC-2; [Pt (*cis*-DACH)(PLA)].

CDDP-treated cultures than in those treated with PC-1, and PC-2. Cultures treated with PC-1 and PC-2 showed a slight increase in glucose half-life compared to the control. In glucose half-life was lengthened after treatment compared with the pretreatment control. The specimens treated with 10^{-4}M of CDDP still exhibited glucose half-life in the post-treatment period (Table 6).

DISCUSSION

Since the antitumor activity of CDDP was first described by Rosenberg et al (1969), it has become an important drug in the treatment of selected human malignant tumors. Approximately 75% of patients with disseminated germ cell tumors are curable with CDDP-based chemotherapy (Burchenal et al, 1979; Kizu et al, 1993)

However, CDDP-induced nephrotoxicity is a major limiting factor for some tumors treated with CDDP. While the unfavorable nephrotoxicity has been overcome by the development of the second-generation agent, carboplatin, there remains an unquestionable need for further improved platinum-containing compounds which have more favorable therapeutic indices and circumvent resistance.

The advanced knowledge of structure-activity relationship of platinum(II) complexes has clarified that the carrier ligands and its leaving groups are essential for their for *in vivo* antitumor activities. The contribution of the carrier ligand may be related to the potency and spectrum of antitumor activity, and that of the leaving group may be related to the dissociation rate from the platinum complex. One of the structural modification that is widely accepted as having resulted in an increased therapeutic index is the attachment of DACH (Connors et al, 1972; Clear & Hoeschele, 1973; Gale et al, 1974; Ridgway et al, 1977; Jung et al, 1998). Several forms of DACH compounds, such as cis-DACH, trans-*l*-DACH, and trans-*d*-DACH, exist. Among these DACH derivatives, trans-*l*-DACH has been known to have more significant antitumor activity (Inagaki & Kidani, 1986). Moreover, it is essential to consider that the leaving group is an important factor influencing the activity of the platinum coordination complexes.

These studies indicated that the dissociation of the leaving groups is an important factor in antitumor and toxic activities of platinum coordination complexes. However, when the rate of dissociation is much higher, it causes toxic effects since the platinum complexes react with normal protein instead of DNA in cancer cell. On the other hand, when the dissociation rate is too low, it is excreted into the extracellular compartment before showing any antitumor activity.

The mechanism of the nephrotoxicity induced by platinum(II) coordination complexes is not completely understood. To date, investigators have demonstrated that cytotoxicity induced by a variety of platinum(II) coordination complexes may be attributed at least in part to the inhibition of blood flow in the kidney or the depletion of intracellular glutathione (Meijer et al, 1982). Dobyan et al (1980) have reported that the site-specific injury was in the pars recta (S3) segment of the proximal tubules. Furthermore, a number of investigator (Jones et al, 1986) suggested that both the proximal and distal tubules have been damaged. The nephrotoxicity of CDDP found in this study is very similar to that reported previously, in terms of both the histopathology and the effects on various measures of renal function (Dobyan et al, 1980; Jones et al, 1986).

The nephrotoxicity induced by platinum(II) coordination complex has been largely abrogated by the routine use of hydration and diuretics (mannitol) and sulfnucleophiles (WR-2721 and diethyldithiocarbamate). It is well documented that mannitol reduces CDDP nephrotoxicity by diluting its tubular urinary concentration rather by altering its half-life, plasma clearance or total urinary excretion (Jones et al, 1986).

The new platinum(II) coordination complex produced and used in this study has generally been screened for antitumor activity and nephrotoxicity using cancer cell lines, HT-1376 and T-24 human bladder carcinoma cells, and rabbit or human renal cortical cells, respectively.

The new synthetic platinum(II) coordination complexes, PC-1 and PC-2, exhibited significant *in vitro* antitumor activity. A criteria for *in vitro* antitumor activity is generally expressed in the cytotoxicity index and is accepted as possible antitumor drugs. PC-1 and PC-2 showed antitumor activity comparable to CDDP. In this study, PC-1 and PC-2 were less cytotoxic than CDDP in renal tissues. It is conceivable that modifications of carrier ligand as a DACH and the leaving group as a PLA derived from CDDP significantly decreased nephrotoxicity.

The appearance of glucose in urine is one of the early sign of proximal tubular dysfunction *in vivo*, and therefore we chose the glucose consumption test as a parameter to assess the nephrotoxicity in human renal cortical tissue. Chang et al (1992) have reported that histocultured renal cortical tissues evaluated using the glucose consumption test provided a good association for CDDP toxicity.

Furthermore, glucose consumption measurements in histocultured human renal cortical tissues were more sensitive than the thymidine-incorporation endpoint (Chang et al, 1994). These results reliable as that of renal cortex because human renal cortical tissues are maintained

tissues are maintained with collagen gel through three dimensional culture method (Chang et al, 1992). The results revealed that the newly developed platinum complex has similar or greater anticancer efficacy compared to CDDP especially high concentration.

Mortine and Borch (1988) reported that the LLC-PK_1 (pig proximal tubule epithelial cell line) was a good model to evaluate the nephrotoxicity induced by CDDP *in vitro*. The present study using primary cultured cells showed reliable data although the LLC-PK_1 cell-line was not used. MTT reduction measurements in normal rabbit kidney cells in monolayer culture and autoradiography on histocultured specimens were also used for the determination of the nephrotoxicity of the new compounds. As mentioned above, however, these new complexes have low nephrotoxicity. Therefore, these new complexes may possible be clinically useful for high-dose chemotherapy with reduced side effects.

Based on these results, these novel platinum complexes represent a valuable lead and justify clinical studies in the development of new anticancer chemotherapeutic agents capable of improving anticancer efficacy with low toxicity.

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