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

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We have synthesized a novel platinum(II) coordination complex containing cis-1,2-diaminocyclohexane (DACH) as a carrier ligand and 1,2-dichloroethane (DCE) as a leaving group. In addition, nitrate was added to improve the water-solubility. A new series of [Pt(cis-DACH)(DCE)] · $2NO_3$(PC) was evaluated for its cytotoxic activity on T-24 and J-82 human bladder carcinoma cells and normal primary cultured kidney cells. PC has demonstrated high levels of cytotoxicity against T-24 and J-82 cells. The cytotoxicity of PC against rabbit proximal renal tubular cells, human renal cortical cells and human renal cortical tissues, determined using the MTT assaying technique, the [^{3}H]-thymidine uptake and glucose consumption tests, was found to be quite less than those of cisplatin. Based on these results, this novel platinum(II) coordination complex appears to be better for improving antitumor activities with low nephrotoxicity and is a valuable lead in the development of new clinically available anticancer chemotherapeutic agents.

Key Words: Selective cytotoxicity, Human bladder carcinoma, Nephrotoxicity, Platinum coordination complex.

INTRODUCTION

The introduction of the square-planar complex cisplatin into the clinical treatment of cancer has resulted in the excellent response rates for some tumor types, especially testicular and ovarian cancers (Rosenberg et al, 1969; Lippman et al, 1973). While the unfavorable nephrotoxicity profile of cisplatin has been overcome by the development of the second-generation agent carboplatin (Calvert et al, 1985; Wiltshaw, 1985), there remains an unquestionable need for few platinum drugs which circumvent acquired cisplatin resistance. As with other cancer chemotherapeutic agents, cellular resistance to the clinically used platinum agents, cisplatin and carboplatin, represents a major clinical limitation to the efficacy (Mangioni et al, 1989; Einhorn, 1990). It is known that renal cortical accumulation of cisplatin lead necrosis of the proximal tubule and late development of internal cysts (Hardaker et al, 1974; Litterest et al, 1977; Krakoff, 1979). The major toxicological limitation is its dose-related accumulative and irreversible side effects of severe nephrotoxicity, nausea and vomiting (Kociba & Sleight, 1971; Schaeppi et al, 1973). Development of resistance in initially responded tumors lead to treatment failure (Andrews & Howell, 1990; Timmer-Bosscha et al, 1992).

The antitumor activity of platinum complexes containing 1,2-diaminocyclohexane (DACH) as a carrier ligand was investigated by Connors et al (1972) and Gale et al (1974). Kidani (1977) synthesized Pt (oxaloto)(trans-*l*-DACH)[1-OPH] and Pt (malonato) (trans-*l*-DACH)[1-PHM] using oxalic acid and malonic acid with selected trans-*l*-DACH among trans-*l*-, trans-*d*- and cis- isomers.

Consequently, there is much interest in obtaining agents that have less toxicity and more favorable anticancer chemotherapeutic indices. To accomplish this aim, we have synthesized a new platinum(II) coordination complex containing DACH as a carrier ligand and 1,2-dichloroethane (DCE) as a leaving group. In

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the present study we evaluated *in vitro* antitumor activity of the new platinum complex on the various human bladder carcinoma cell lines and its cytotoxicity on rabbit proximal tubular cells, human renal cortical cells and on 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 chemicals were purchased from Sigma chemical Corp. (St. Louis, MO). Powdered medium, EDTA-trypsin and soybean 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 & 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 of Pt(II)(cis-DACH)(DCE)($2NO_3$)(Fig. 1); the PC utilized was 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

Primary rabbit kidney proximal tubular cells

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). Primary cultures were initiated from purified rabbit kidney proximal tubules by a modification of the method described by Chung et al (1982) (Jung et al, 1992; Taub, 1997). To summarize, each kidney was perfused via the renal artery, first with PBS to remove the blood, and then with a 0.5% (w/v) solution of iron oxide. The renal cortex was removed by slicing and the renal cortical slices were homogenized in a sterile Dounce homogenizer. The tubules in the homogenate were then filtered through a 253 μ mesh screen, and then through an 83 μ mesh screen. The material which remained on the 83 μ mesh (proximal tubules and glomeruli)was transferred to a 50 ml tube containing basal medium (DME/F12 medium) The glomeruli (which were covered with iron oxide) were then removed with a stir bar. The remaining material, the renal proximal tubules, was incubated for two minutes at 23°C in basal medium containing 0.125 mg/ml collagenase (class IV) and 0.025% soybean trypsin inhibitor. The tubules were washed by centrifugation, and the primary cultures were inoculated into 35 mm culture dishes containing basal medium further supplemented with 5 μg/ml insulin, 5×10^{-8} M hydrocortisone and 5 μg/ml transferrin. Approximately 100 culture dishes of proximal tubule cells could be obtained from a single rabbit kidney preparation. The cultures 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 remove unattached tubules. The medium was replaced every two days thereafter. During the initial 4 days, cell outgrowth occurred from the attached tubules in culture.

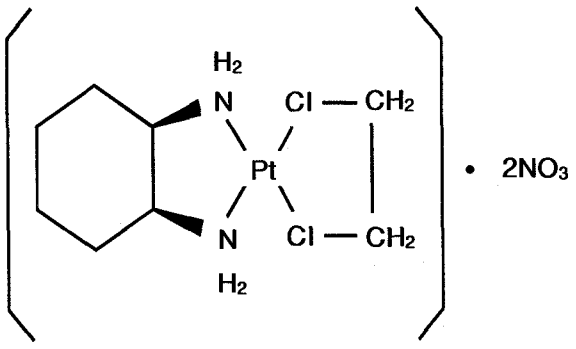

Fig. 1. Structure of a new platinum(II) coordination complex. DCE: 1,2-Dichloroethane, DACH: 1,2-Diaminocyclohexane

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 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 (1,000 rpm for

5 minutes) and the particles of the kidney cortical tissues were suspended in DME/F12 medium supplemented with insulin (5 μg/ml), transferrin (5 μg/ml), hydrocortisone (5×10^{-8} M), triiodothyronine (5 μg/ml), prostaglandin E_2 (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 experiments.

Cancer cell lines

Human bladder carcinoma cell lines (T-24 and J-82) were obtained from Cancer Research Center, Seoul National University, Seoul, Korea. Human bladder carcinoma cells were cultured in RPMI 1640 medium supplemented with 10% FBS, 20 mM sodium bicarbonate, 15 mM HEPES, 92 units/ml penicillin G and 200 μg/ml streptomycin. All the cells were incubated in humidified 5% CO_2/95% air at 37°C.

Histoculture

Normal kidney tissues, identified by frozen section at the time of radical nephrectomy, were transported in a container to the laboratory which is near the operating room. The normal human kidney tissues were divided into 2~3 mm diameter pieces and five pieces were placed on the top of each previously hydrated Spongostan gel (1×1×1 cm) (Health Design Industries, Rochester, NY). One gel was put in each well of a six-well plate. 3 ml of Eagle's minimal essential medium (MEM) supplemented with 10% FBS, 50 μg/ml gentamicin and 1 μg/ml cefotaxime were added to each well of the plate. The final volume of the medium was sufficient to reach the upper gel surface without immersing it. Culture plates were covered and maintained in humidified 5% CO_2/95% air atmosphere at 37°C. The histoculture was incubated in medium containing 50 μM of newly synthesized platinum(II) coordination complex and cisplatin, and the specimens were washed with PBS and transferred to fresh medium (Freeman & Hoffman, 1986; Chang et al, 1992).

In vitro antitumor activity

Cancer cell lines were cultured in the growth media for each cell 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 0.1 ml of the appropriate medium containing 10^5 cells. New platinum(II) coordination complex and cisplatin were added in various concentrations. 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 were added to each well and absorption rates at 630 nm 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 concentrations: 5, 15, 50, 150, 300, 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 630 nm. All experiments were performed at least 3 times, with 6 wells for each concentration of the new platinum(II) coordination complex 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 fo 10^5 cells per well. After 1 hour 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 5,000 TD).

Glucose consumption by histocultured specimens.

This study was performed essentially as described by Chang et al (1992). In brief, 50 μl of culture medium was 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 content of the medium plotted in semilog form against time using the Sigma plot program (Janel Scientific, Corte, Madera, CA). A simple exponential model of glucose consumption was then fitted to the data with the Systat program (Systat Inc, Evanston, IL). The half-life of glucose was calculated from the slope parameter of this model using the equation t=0.693/s, where s=slope of the best fit linear regression line or the natural log of the glucose concentration plotted against time. The glucose content of the medium was measured daily for 3 days. The log values over 3 days were plotted against time and the slope of the best-fit line was taken as a glucose consumption rate during the 3-day measurement period (one period).

RESULTS

Selective cytotoxicity

The sensitivity of the T-24 and J-82 human bladder cancer cell-lines to the 2 platinum-containing compounds are shown in Table 1. Drug concentration (CC_{50}), cytotoxic index by 50%, was determined through MTT assaying after treatment with the compounds for 48 hours. CC_{50} of cisplatin for the T-24 and J-82 cells did not differ significantly.

To investigate renal cytotoxicity, primary cultured rabbit and human normal kidney cells were incubated with various concentrations of cisplatin and the new compound for 48 hours. The cytotoxicity of PC on primary cultured normal kidney cells was lower, by about 7- and 9-fold, compared with cisplatin (Table 2 and 3).

Effect on [^{3}H] thymidine uptake by normal kidney cells

To evaluate the nephrotoxicity of the new Pt(II)

Table 1. *In vitro* cytotoxicities of Pt(II) complexes on T-24 and J-82 human bladder carcinoma cells

Compounds	CC_{50} (μM)	
	T-24	J-82
Cisplatin	32.5±5.24[a)]	59.6±7.50
PC[b)]	46.3±4.78	68.2±8.49

CC_{50} indicates mean cytotoxic concentration with MTT assay, [a)]Each value is the mean±S.E of three experiments, [b)][Pt(II)(cis-DACH)(DCE)]2NO_3

Table 2. *In vitro* cytotoxicities of platinum complexes on proximal tubular cells of rabbit kidney

Compounds	CC_{50} (μM)	Ratio[a)]
Cisplatin	22.7±3.51[b)]	–
PC[c)]	214.3±25.92*	9.44

a) The ratio is the CC_{50} of the PC divided by that of the cisplatin, b) Each value is the mean±S.E of at least three experiments, c) [Pt(II)(cis-DACH)(DCE)] · 2NO_3. *Significantly different from cisplatin-control ($p<0.05$)

Table 3. *In vitro* cytotoxicities of platinum complexes on cortical cells of human kidney

Compounds	CC_{50} (μM)	Ratio[a)]
Cisplatin	27.5± 4.32[b)]	–
PC[c)]	194.6±22.68*	7.08

a) The ratio is the CC_{50} of the PC divided by that of the cisplatin, b) Each value is the mean±S.E of at least three experiments, c) [Pt(II)(cis-DACH)(DCE)] · 2NO_3. *Significantly different from cisplatin-control ($P<0.05$)

Table 4. Effect of platinum(II) coordination complexes on ^{3}H-thymidine in corporation into primary cultured proximal tubular cells of rabbit-kidney

Compounds	[^{3}H]-thymidine uptake	Uptake rate (%)
Control	617.2±74.26	100.0
Cisplatin	21.4 ± 5.75	3.5
PC	324.7±36.08	52.6

Concentration of platinum(II) coordination complexes in culture medium; 5×10^{-5} M, PC: [Pt(II)(cis-DACH)(DCE)$(NO_3)_2$]. Values are means±S.E. All the incorporations were determined in triplicate.

Table 5. Effect of platinum(II) coordination complexes on ^{3}H-thymidine incorporation into primary cultured renal cortical cells of human-kidney

Compounds	[^{3}H]-thymidine uptake	Uptake rate (%)
Control	633.5±81.45	100.0
Cisplatin	27.0± 5.36	4.3
PC	341.8±41.24	54.0

Concentration of platinum(II) coordination complexes in culture medium; 5×10^{-5} M, PC: [Pt(II)(cis-DACH)(DCE)$(NO_3)_2$]. Values are means±S.E. All the incorporations were determined in triplicate.

complex, we measured [^{3}H] thymidine uptake by rabbit proximal tubular cells and human renal cortical cells.

Rabbit renal proximal tubular cells

The cytotoxicities of cisplatin and PC against primary cultured rabbit renal proximal tubular cells are shown in Table 2. PC (CC_{50}: 214.3 μM) showed a lower level cytotoxicity compared to cisplatin. In addition to the MTT assay technique, cytotoxicity was determined using [^{3}H] thymidine uptake assay technique. Results using this assaying technique are shown in Table 4. At a concentration of 5×10^{-5} M cells treated with PC showed a [^{3}H] thymidine uptake of 52.6%, whereas those treated with cisplatin showed a value of 3.5%. These results indicate that the cytotoxicity of PC was significantly less than that of cisplatin, and the ^{3}H] thymidine uptake assay is more sensitive than the MTT assay.

Human renal cortical cells

PC showed significantly lower cytotoxicity (CC_{50}: 194.6 μM) than cisplatin (CC_{50}: 27.5 μM) (Table 3). Table 5 shows that [^{3}H] thymidine incorporation is more significantly inhibited by cisplatin (95.7%) than by PC (46.0%).

Effect on glucose consumption by human kidney tissues

The glucose consumption rate was calculated as the half-life of the medium glucose content. The medium glucose half-life varied with the drug tested and with the drug concentration (Fig. 2). In measuring the

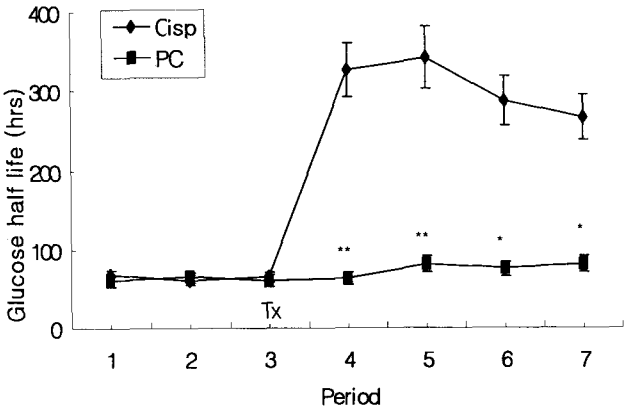

Fig. 2. Change of glucose half-life on histocultured human renal cortical tissue speciemens that were treated with 10^{-4} M cisplatin and 10^{-4} M experimental drug (PC) for 72 hours exposure. One period constitutes 3 days. All experimental histocultures had their own control that was pretreatment three period. Glucose half-life on control speicimen was very steady. Tx: treatment, Cisp: cisplatin, PC: [Pt(II)(cis-DACH)(DCE)]2NO_3. *Significantly different form cisplatin - control (*P<0.05, **P<0.01)

glucose consumption, one period was defined as more than 3 measurements per day in a 4-week culturing period of human renal cortical tissue. The glucose half-life before adding PC was approximately 60~64 hours and did not show any significant increase for 4 periods, and then increased to over 80 hours from the fifth period up to the 7th period. However, the effect of PC was less marked than that of cisplatin. The half-life of the medium glucose content was longer in cisplatin-treated cultures than in those treated with PC. Cultures treated with PC showed a slight

increase in glucose half-life compared to the control. In the case of cisplatin, the glucose half-life was lengthened after treatment compared with the pre-treatment control. The specimens treated with 10^{-4} M of cisplatin still exhibited prolonged glucose half-life in the post-treatment period.

DISCUSSION

Cis-diaminedichloroplatinum II (cisplatin) was found to be the most active among platinum(II) coordination complexes in experimental tumor systems and has proven to be of clinical value (Rosenberg et al, 1967, 1969). Since Rosenberg et al (1969) first described the antitumor activity of cisplatin, cisplatin has become an important drug in the treatment of selected human malignant tumors. However, its clinical uses are limited by its dose-related renal toxicity. 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 has clarified that the carrier ligands and its leaving groups are essential for their 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 modifications 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, 1999). 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. The platinum (II) coordination complexes appear to penetrate cell membranes by diffusion, and then the leaving group is displaced directly by hydrolysis with nucleic acid groups. This is responsible for the formation of activated species of platinum(II) coordination complexes which react with DNA(i.e., with the guanine N7 forms), causing the inhibition of DNA replication and cytotoxic effects (Tashiro, 1988). In addition to their reactivity with DNA, platinum(II) coordination complexes can react with protein-bound sulfhydryl groups of the renal proximal tubules, resulting in significant toxic effects on renal function (Alden & Repta, 1984; Appleton et al, 1989). These studies indicated that the dissociation of the leaving groups is an important factor in antitumor and toxic activities. 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 cells. 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. Gonzalez-Vitale et al (1980) noted that the distal tubule was the most consistently damaged region in the human kidney. Furthermore, a number of investigators (Jones et al, 1986) suggested that both the proximal and distal tubules have been damaged. The nephrotoxicity of cisplatin 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 effects of different doses of cisplatin on the kidney have been published (Litterst, 1981; Daley & McBrien, 1992).

The relatively modest level of nephrotoxicity associated with the administration of high doses of cis-$[Pt(NH_3)_2(guanosine)_2]^{2+}$ as compared with the other platinum complexes investigated suggests that analogous compounds resulted from the repair of platinated DNA which will not produce reactive species that exacerbate the nephrotoxicity arising from the administration of the parent compound. Such repair products could be expected to be released slowly when the characteristic cisplatin-DNA lesions are repaired. Since this compound dose not readily undergo substitution processes in which the guanosine is removed (Jones

& Beaty, 1991), the renal retention of platinum following its administration may be dependent on the occurrence of ligand substitution processes in which one or more of the ammonia groups bound to the platinum are replaced by groups such as sulfur molecules that are present in the sulfhydryl groups of proteins. An alternative explanation is that the metabolism of the coordinated guanosine may be responsible for the retention of platinum-containing species in the renal tissue (Rosenberg, 1978; Daley & McBrien, 1982, 1984).

The new platinum(II) coordination complex produced and used in this study has generally been screened for antitumor activity and nephrotoxicity using human bladder carcinoma cell lines and normal human and rabbit kidney cells, respectively. The new synthetic platinum(II) coordination complex, PC, exhibited significant *in vitro* antitumor activity against human bladder carcinoma cell lines.

A criteria for *in vitro* antitumor activity is generally expressed in the cytotoxicity index and is accepted as possible antitumor drugs. PC showed antitumor activity comparable to cisplatin. The comparison of antitumor activities of PC and cisplatin exhibited similarity in levels of activity against T-24 and J-82 human bladder cancer cell lines. Since the only difference in chemical structure between PC and cisplatin is the "DACH-isomer", it can be inferred that the dissociation times or rates are different after absorption of the compounds in the cells due to the different leaving groups.

In this study, PC was less cytotoxic than cisplatin in renal tissues. It is conceivable that the modifications of the carrier ligand as a DACH and the leaving group as a DCE derived from cisplatin significantly decreased nephrotoxicity. Mortine & Borch (1998) reported that the LLC-PK_1 (pig proximal tubule epithelial cell line) was a good model in evaluating the nephrotoxicity induced by cisplatin *in vitro*. My present study using primary cultured cells showed reliable data although the LLC-PK_1 cell line was not used.

In vivo, the appearance of glucose in urine is one of the early signs of proximal tubular dysfunction, 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 cisplatin toxicity. Furthermore, glucose consumption measurements in histocultured human renal cortical tissues were more sensitive than the thymidine-incorporation endpoint (Chang et al, 1994). The results revealed that the newly developed platinum complex has similar or greater anticancer efficacy compared to cisplatin, especially high concentrations. As mentioned above, however, this new complex has very low nephrotoxicity. Therefore, this new complex may possibly be clinically useful for high-dose chemotherapy with reduced side effects.

Based on these results, this novel platinum complex represents a valuable lead and justifies clinical studies in the development of a new anticancer chemotherapeutic agent capable of improving anticancer efficacy with low toxicity.

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REFERENCES

Alden MW, Repta AJ. Exacerbation of cisplatin-induced nephrotoxicity by methionine. *Chem Biol Interact* 48: 121−127, 1984

Andrews PA, Howel SB. Cellular pharmacology of cisplatium: perspectives on mechanisms of acquired resistance. *Cancer Cells* 2: 35−43, 1990

Appleton TG, Bennett MA, Tomkins IB. Effect of chelatering size on spectroscopic and chemical properties of methylplatinum(II) complexes of the ditertiary phosphines Ph2P[CH2]nPh2 (n=1,2or3). *JCS Dalton* 439−446, 1976

Calvert AH, Harland SJ, Newell DR, Harrap KR. Phase I studies with carboplatin at the Royal Marsden Hospital. *Cancer Treat Rev* 12: 51−57, 1985

Chang SG, Slocum HK, Torth K, Hoffman RM. Glucose consumption end point in primary histoculture indicates recovery of human tumors. *Anticancer Res* 14: 77−84, 1994

Chang SG, Toth K, Black JD, Slocum HK, Perrapato SD, Huben RP, Rustum YM. Growth of human renal cortical tissues on collagen gel. *In Vitro Cell Dev Biol* 28A: 128−135, 1992

Chung SD, Alavi N, Livingston D, Hiller S, Taub M. Characterization of primary rabbit kidney cultures that express proximal tubule functions in a hormonally defined medium. *J Cell Biol* 95: 118−126, 1982

Clear MJ, Hoeschele JD. Antitumor platinum compound: relationship between structure and activity. *Platinum Metals Review* 17: 2−10, 1973

Connor TA, Jones M, Ross WCJ. New platinum complexes with antitumor activity. *Chem Biol Interact* 5: 415−420, 1972

Cook WF, Pickering GW. A rapid method for separating glomeruli from rabbit kidney. *Nature* 182: 1103−1104, 1958

Daley-Yates PT, McBrien DCH. The inhibition of renal ATPase by cisplatin and some biotransformation product. *Chem Biol Interact* 40: 325−330, 1982

Daley Yates PT, McBrien DCH. Cisplatin metabolites in plasma, a study of their pharmacokinetics and importance in the nephrotoxic and antitumor activity of cisplatin. *Biochem Pharmacol* 33: 3603−3069, 1984

Dobyan DC, Levi J, Jacobs C. Mechanism of cisplatinum nephrotoxicity. *J Pharmacol Exp Ther 213*(3): 515−556, 1980

Einhorn LH. Treatment of testicular cancer: A new and improved model. *J Clin Oncol* 8: 1777−1781, 1990

Einhorn LH, Donohue J. Cis-diamminedichloroplatinum, vinblastine and bleomycin combination chemotherapy in disseminated testicular cancer. *Ann Intern Med* 87: 293−328, 1977

Freeman AE, Hoffman RM: *In vivo*-like growth of human tumors *in vitro. Proc Natl Acad Sci* USA 83: 2694−2698, 1986

Gale GR, Walker EM, Atkins LM. Antileukemic properties of dichloro (1,2 diaminocyclohexane) platinum (II) *Res Commun Chem Pathol Pharmacol* 7: 529−533, 1974

Gonzales-Vitale JC, Hayes DM, Sternberg SS. The renal pathology in clinical trials of cisplatinum. *Cancer* 39: 1362−1371, 1977

Hardaker WT, Stone RA, McCoy R. Platinum toxicity. *Cancer* 34: 1030−1035, 1974

Inagaki K, Kidani Y. Differences in binding of (1,2-diaminocyclohexane) platinum(II) isomers with d (GPG). *Inorg Chem* 25: 1−8, 1986

Jones MM, Basinger MA, Craft WD. Control of some aspects of cis-platinum nerphrotoxicity. *Arch Toxicol* 59(3): 167−171, 1986

Jones MM, Beaty JA. Rate of displacement of guanosine by cyanide from cis-diammine bis (guanosine) platinum (II) chloride determined by using [^{13}C]-NMR spectroscopy. *Inorg Chem* 30: 1584−1592, 1991

Jung JC, Kim YK, Park SJ, Chang SG, Lee KT, Baek MS, Park JJ, Rho YS. *In vitro* cytotoxicity of normal platinum(II) coordination complexes containing diaminocyclohexane and diphenylphosphines. *Korean J Physiol Pharmacol* 2: 395−401, 1998

Jung JC, Kim YK, Yim SV, Park SJ, Chung JH, Chang SG, Lee KT, Rho YS. Selective cytotoxicity of a novel platinum(II) coordination complex on human gastric cancer cell lines and normal kidney cells. *Korean J Physiol Pharmacol* 3: 283−291, 1999

Jung JC, Lee SM, Kadakia N, Taub M. Growth and function of primary rabbit kidney proximal tubule cells in glucose-free serum-free medium. *J Cellular Physiol* 150: 243−250, 1992

Kidani Y. Development of antitumor platinum complexes. *Yakugaku Zasshi* 105(10): 909−914, 1985

Krakoff IH. Nephrotoxicity of cis-diamminedichloroplatinum(II). *Cancer Treat Rep* 63: 1523−1529, 1970

Lippman AJ, Helson L, Krakoff IH. Clinical trials of cis-diammine dichloroplatinum (NSC-119875). *Cancer Chemother Rep* 57: 191−200, 1973

Litterst CL. Alterations in the toxicity of cis-dichlorodiammineplatinum(II) and in tissue localization in the. vehicle of administration. *Toxicol Appl Pharmacol* 61: 99−104, 1981

Litterest CL, Torres IJ, Guarino AM. Plasma levels and organ distribution of platinum in rat, dog, and fish following intravenous administration of cis - DDP(II). *J Clin Hematol Oncol* 7: 169−178, 1977

Mangioni C, Bolis G, Pecorelli S, Bragman K, Epis A, Favalli G, Marsoni S. randomized trial in ovarian cancer comparing cisplatin and carboplatin. *J Natl Cancer Inst* 81: 1464−1468, 1989

Meijer S, Mulder NH, Sleijfer DT, Schraffordt KH. Neprotoxicity of cisplatinum during remission-induction and maintenance chemotherapy of testicular carcinoma. *Cancer Chemother Pharacol* 8(1): 27−30, 1982

Mortne TJ, Borch RF. Quiescent LLC-PK cells as a model for cis-daminedichloro platinum(II) nephrotoxicity and modulation by thio rescue. *Cancer Res* 48: 6017: 24−29, 1988

Mossman T. Rapid colorimetric assay for cellular growth and survival: application and cytotoxic assays. *J Immumol Methods* 65: 55−61, 1973

Ridgway HJ, Speer RJ, Hall JM. Analogs of sulfato 1,2-diaminocyclohexane platinum (II): Modifidcations inleaving ligands. *J Clin Hematol Oncol* 7: 220−227, 1977

Rosenberg B. Platinum complex-DNA interactions and anticancer activity. *Biochimie* 60: 399−406, 1978

Rosenberg B, Van Camp L, EB, Thomson AJ. The inhibition of growth or cell division in Escherichia coli by differernt ionic species of platinum complexes. *J Biol Chem* 242: 1347−1352, 1967

Rosenberg B, Van Camp L, Trosko JE, Mansour VH. Platinum compounds: A new class of potent antitumor agents. *Nature* 222: 385−386, 1969

Shimoyama Y, Kubota T, Watanabe M Predictability of *in vivo* chemosensitivity by *in vitro* MTT assay with reference to the clonogenic assay. *J Surg Oncol* 41: 12−18, 1989

Tashiro T. Antitumor activities and mechanism of platinum(II) complexes. *Jap J Chem* 4: 684, 1988

Taub M. Primary kidney cells, in *Methods in Molecular Biology* 75: Basic Cell Culture Protocols (JW Pollard and JM Walker, Eds) Humana press, inc., Totowa NJ p 153−161, 1997

Timmer-Bosscha H, Mulen NH, DeVries EGE. Modulation of cis-diaminedichloroplatinum(II) resistance, a review. *Br J Cancer* 66: 227−238, 1992

Wiltshaw E. Ovarian trials at the Royal Marsden. *Cancer Treat Rev* 12: 67−71, 1985