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

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We have synthesized novel platinum (II) coordination complex containing cis-1,2-diaminocyclohexane (DACH) as a carrier ligand and 1,2-bis(diphenylphosphino)ethane (DPPE) as leaving group. Furthermore, nitrate was added to improve the water-solubility. A new series of [Pt(cis-DACH)(DPPE)] · $2NO_3$ (PC) was evaluated its antitumor activity on various MKN-45 human gastric adenocarcinoma cell-lines and normal primary cultured kidney cells. The new platinum complex demonstrated high efficacy in the cytotoxicity on MKN-45 cell-lines as well as adriamycin-resistant (MKN-45/ADR) and cisplatin-resistant (MKN-45/CDDP) cells. The cytotoxicities of PC were found quite less than those of cisplatin in rabbit proximal renal tubular cells, human renal cortical cells and human renal cortical tissues using MTT assay, [^{3}H]-thymidine uptake and glucose consumption tests. Based on these results, this novel platinum (II) coordination complex, was considered as better a valuable lead for improving antitumor activities with low nephrotoxicities in the development of a new clinically available anticancer chemotherapeutic agents.

Key Words: Selective cytotoxicity, Nephrotoxicity, Platinum coordination complex

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum II) is one of the first line chemotherapeutic agents for the treatment of ovarian carcinoma, testicular cancer, bladder cancer and cancer of the head and neck (Hill et al, 1975; Gottlieb & Drewinko, 1975; Einhorn & Donohue, 1977; Harstrick et al, 1989). The platinum (II) coordination complexes are cytotoxic agents that were first identified by Rosenberg and coworkers in 1965. Growth inhibition of *E. coli* was observed when electrical current was delivered between platinum electrodes. The introduction of the square-planar complex cisplatin has resulted in dramatic improvement in the response rate for some tumor types, notably testicular tumor and ovarian carcinoma (Rosenberg, 1978; Hamilton et al, 1985).

While the unfavorable toxicity profile of cisplatin (primary nephrotoxicity) has been overcomed 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 (Harstrick et al, 1989; Ettinger et al, 1994; Fields et al, 1994). It was also known that renal cortical accumulation of cisplatin leads to the necrosis of the proximal tubule and late development of internal cysts (Hardaker et al, 1974; Krakoff, 1979; Litterest, 1981). To date, there have been notable investgations for novel platinum chemistry adressing their stabilities, broad antitumor activities, and lower nephrotoxicities. The antitumor activities of platinum (II) coordination complexes containing diaminocyclohexane (DACH) as a carrier ligand were investigated by Connors (1972), Clear (1973), and Gale (1974). Kidani (1985) also synthesized [Pt (II) (oxalato) (trans-*l*-DACH) [*l*-OHP] and [Pt (II) (malonato) (trans-

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l-DACH)] [*l*-PHM] using oxalic acid/malonate 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 reported the sythesis of a new platinum (II) coordination complex containing DACH as a carrier ligand and 1,2-bis(diphenylphosphino) ethane (DPPE) as a leaving group.

In the present study we evaluated *in vitro* antitumor activity of the new platinum complex on the various human gastric adenocarcinoma cell-lines and its nephrotoxicities on the rabbit proximal tubular cells, human renal cortical cells and histocultured human renal cortical tissues.

METHODS

Materials

Dulbeco's Modified Eagle's (DME), 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 obatained from Worthington Co. (Freehold, NY). Iron oxide was prepared by the method of 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 use Pt (II) (cis-DACH) (DPPE) ($2NO_3$); PC was synthesized in the Department of Pharmacochemistry, College of Pharmacy, Kyung Hee University as previously reported (Jung et al, 1995). This agent was dissolved in sterilized cell culture media prior to use.

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 of 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 (253 μ mesh screen, and then through an 83 μ 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). The glumeruli (which were coverd with iron oxide) were then removed with a stir bar. The remaining material, 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 tubular 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 during the day of plating. The day after plating, the medium was replaced so as to remove unattached tubules. The medium was replaced every two days thereafter. During the initial 4 days in culture, cell outgrowth from attached tubules occurred.

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 DMF/F12 (1 : 1) medium supplemented with penicillin G/streptomycin. A single-cell sespension was obtained by mechanical disaggregation with a srerilized 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 media 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 in an

incubator at 37°C maintaining a highly humidified atmosphere 5% CO_2/95% air. After 2 weeks of incubation, the cells were confluent and used for experiments.

Cancer cell-lines: MKN-45 human gastric adenocarcinoma cell-lines (MKN-45/S, MKN-45/ADR and MKN-45/CDDP) were obtained from Cell-Biology Lab. of Atomic Power Hospital, Seoul, Korea. MKN-45 cells were cultured in RPMI 1,640 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 5% CO_2/95% air in a humidified incubator at 37°C.

Histoculture: Normal kidney tissues, identified by frozen section the time of radical nephrectomy, were transported in a container to the laboratory which was near the operating room. The normal human kidney tissues were devided into 2 to 3 mm diameter pieces and five pieces were placed on the top of previously hydrated Spongostan gel (1×1×1 cm) (Health Design Indust. Rochester, NY). One gel was put in each well of six-well plate. 3 ml of Eagle's minimal essental 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 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. Histoculture was incubated in medium containing 50 μM of newly formed platinum (II) coordination complexes and cisplatin, the specimens were washed with PBS and transferred to fresh media (Freeman & Hoffman, 1986; Chang et al, 1992).

In vitro antitumor activity

Cancer cell-lines were cultured in the growth media for each cell lines in an incubator maintaining a highly humidified 5% CO_2/95% air at 37°C. After 3 days culture, 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 96-well tissue culture microtiter plate were inoculated with 0.1ml of the appropriate media containing 10^5 cells. New platinum (II) coordination complex and cisplatin were added at 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) were added to each well. After 4 hours of exposure, 0.05 ml of DMSO were added to each well and absoption rates were read at 630 nm by an ELISA reader (Shimoyama et al, 1989).

Cytotoxicities 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 resuspending in DME/F12 medium. This suspension was seeded at 10^5 cells per well on 96-well plate in 100 μl of medium. Drugs were added at various concentrations (final concentration; 5, 15, 50, 150, 300 and 500 μM) and the cell cultures were incubated for 48 hours in an incubator maintaining a highly humidified atomosphere of 5% CO_2/95 air at 37°C. There after, 50 μl of the medium containing MTT (5 mg/ml) was removed and the wells were washed with PBS, and then of 50 μl of DMSO were added to each well to solublize the precipitates. Then the plates were transferred to an ELISA reader to measure the extracted dye at 630 nm. All experiments were performed at least 3 times, with 6 wells for each concentration of new platinum (II) coordination complex and cisplatin (Mossman, 1973).

Thymidine uptake

Primary cultured proximal tubular and cortical cells were respectively seeded at the cell density of 10^5 per well in 24 well plate. After 1 hour of incubation, platinum (II) coordination complexes were added and incubated for 48 hours under a humidified incubator, 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 0.5 M-NaOH for 2 hours at 37°C. The amount of radioactivity uptake by the cells was determined by neutralizing with 0.5 M-HCl, adding scintillating cocktail (Scint-AXF, Packard, CT) and counting in a β-counter (Beckman, LS 5,000 TD).

Glucose consumption from histocultured specimens

This study was performed essentially as described by Chang et al (1992). 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 catalized by hexokinase with the glucose content of the medium was plotted in semilog from *versus* time using the Sigma plot program (Jandel Scienfic, 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 of the natural log of the glucose concentration plotted *versus* time. The glucose content of the medium was measured daily for 3 days. 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

Selective cytotoxicity

The sensitivities of the various MKN-45 human gastric cancer cells, wild type, a resistant phenotype to adriamycin (MKN-45/ADR) and a resistant phenotype to cisplatin (MKN-45/CDDP) to the 2 platinum containing compounds are shown in Table 1. Drug concentration (CC_{50}), cytotoxic index by 50%, was determined through MTT assay after treatment with compounds for 48 hours. Cisplatin did not show significantly different CC_{50} between MKN-45/S and MKN-45/ADR cells, whereas the a new compound (PC) showed 2-fold resistance against MKN-45/ADR cells. On MKN-45/CDDP cell-line with acquired cisplatin resistance, cytotoxicity of cisplatin was prominently weak compared with other type cells, while new compounds showed much higher cytotoxicity. To investigate renal cytotoxicity, primary cul-

Table 1. *In vitro* cytoxicities of Pt (II) complexes on MKN-45/S, MKN-45/ADR and MKN-45/CDDP cells

Compound	CC_{50} (μM)		
	MKN-45/S[a)]	MKN-45/ADR[b)]	MKN-45/CDDP[c)]
Cispatin	27.4±3.43[d)]	22.8±1.86	149.5±15.67
PC	32.1±3.05	65.3±6.72*	61.0±7.14

CC_{50} indicates mean cytotoxic concentration with MTT assay, a) wild type cell, b) adriamycin-resistant cell, c) cisplatin-resistant cell, d) Each value is the mean±S.E. of three experiments. PC: Pt (II) (cis-DACH) (DPPE) · $2NO_3$
*Significantly different from cisplatin-control ($P<0.05$)

Table 2. *In vitro* cytotoxicities of Pt (II) complexes on proximal tubular cells of the rabbit kidney

Compound	CC_{50} (μM)	Ratio[a)]
Cisplatin	14.8±5.36[b)]	–
PC	187.5±17.52	12.7

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

Table 3. *In vitro* cytotoxicities of Pt (II) complexes on cortical cells of human kidney

Compound	CC_{50} (μM)	Ratio[a)]
Cisplatin	17.2±2.73[b)]	–
PC	225.4±19.18	13.1

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.

tured rabbit and human normal kidney cells were incubated with cisplatin and new compounds for 48 hours in various concentrations. The cytotoxicity of PC on primary cultured normal kidney cells was markedly lower 12.7 and 13.1-fold compared with cisplatin (Table 2 & 3).

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

To evaluate the nephrotoxicity of new Pt (II) complex, we measured [^{3}H] thymidine uptake in 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}: 187.5 μM) showed less as compared with cisplatin. In addition to MTT assay, cytotoxicities were determined using [^{3}H] thymidine uptake assay. Results using this assay are shown in Table 4. PC showed 59.2% of [^{3}H] thymidine uptake as compared with that of cisplatin (1.5%) at 500 μM. These result indicate that cytotoxicity of PC was significantly less than that of cisplatin, and [^{3}H] thymidine uptake assay is more sensitive than MTT assay.

Human renal cortical cells: PC showed significantly less cytotoxicity (CC_{50}: 225.4 μM) than that of cisplatin (CC_{50}: 17.2 μM) (Table 3). Table 5 shows that [^{3}H] thymidine incorporation is significantly inhibited by cisplatin (1.4%) as compared with that of PC (42.3%).

Glucose consumption on human kidney tissues

The glucose consumption rate was calculated as the medium glucose-content half-life. The medium glucose half-life varied with two the different drugs tested, depending on the drug concentration (Table 6).

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

Compound	[^{3}H]-thymidine uptake (cpm/10^5 cells)	Uptake rate (%)
Control	598.3±75.15	100.0
Cisplatin	9.0±3.46	1.5
PC	354.3±42.68	59.2

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

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

Compound	[^{3}H]-thymidine uptake (cpm/10^5 cells)	Uptake rate (%)
Control	621.3±56.01	100.0
Cisplatin	8.7±5.14	1.4
PC	263.0±60.98	42.3

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

Table 6. Nephrotoxicity on histocutured human kidney tissues by glucose consumption assay

Compound	Glucose Half-Life (hours, M±S.D.)						
	1	2	3	4	5	6	7 (period)
Cisplatin	63.3±6.02	59.2±2.69	59.2±4.86	312.6±41.30	328.4±38.06	246.4±34.66	242.0±32.98
PC	52.1±3.67	49.7±4.49	49.8±3.28	55.6±2.38*	74.5±6.89**	70.3±7.29*	74.7±8.32*

The histocultured human kidney tissues treated with 10^{-4} M cisplatin and 10^{-4} M PC with 72 hours exposure. All experimental histocultures had their own control (period 1, 2, 3). The glucose $T_{1/2}$ was abruptly delayed after treatment with PC and was not recovered. In contrast, the glucose $T_{1/2}$ was gradually increased and recovered in the case of cisplatin-treated histocultures. PC: [Pt (II) (cis-DACH) (DPPE)] · $2NO_3$. *Significantly different from cisplatin-control (*$P<0.001$)

In glucose consumption, one period is defined as more than 3 times measurement per day in 4 week-culture of human renal cortical tissue. The half-life of glucose before adding PC is approximately 50~52 hours and dose not show any significant increase at 4 period, and then showed more than 75 hrs at 5~7 periods. However, the effect of PC was less marked than that of cisplatin. The medium glucose content half-life was longer in cisplatin-treated cultures than those of PC. PC showed slight increase in glucose half-life compared to 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 had delayed glucose half-life in the post-treatment period.

DISCUSSION

Cis-diaminedichloroplatinum II (cisplatin) was found to be the most active one 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 (1967) 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 overcomed 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 on structure-activity relationship was 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 those leaving group may be related to the dissociation rate from 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; Cleare & Hoeschele, 1973; Gale et al, 1974; Ridgway et al, 1977). Several DACH compounds are existed such as the forms of such as cis-DACH, trans-*l*-DACH and trans-*d*-DACH. Among these DACH derivatives, trans-*l*-DACH has been known to have more significant antitumor activity (Inagaki & Kitani, 1986).

Moreover, it is essential to consider that the leaving group is an important factor influencing the activity of platinum coordination complexes. The platinum (II) coordination complexes appear to penetrate cell membrane 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 reacts with DNA (i.e., with the guanine N7 forms), causing the inhibition of DNA replication and cytotoxic effect (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 with resulting in significant toxic effect on renal function (Alden & Repta, 1984; Appleton et al, 1989). These studies indicated that the dissociation of leaving group is an important factor in antitumoral 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. Contrastly, when the dissociation rate is too low, it is excreted to extracellular compartment before showing any antitumor activity.

The mechanism of nephrotoxicity induced by platinum (II) coordination complexes is not completely understood. To date, investigators have demonstrated that cytotoxicity induced by a variety of platinum coordination complexes may be attributable at least in part to the inhibition of blood-flow in kidney or depletion of intracellular glutathione(Meijer et al, 1982). Dobyan et al (1980) have reported that the site-specific injury was 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 human kidney. Furthermore, a number of investigators (Porter et al, 1981; Jones et al, 1985) suggested that both of proximal and distal tubules have been damaged. The nephrotoxicity of cisplatin found in this study is very similar to that of reported previously, in terms of both the histopathology and the effects on various measures of renal function (Dobyan et al, 1980; Jones et al, 1985). The effects of different doses of cisplatin on kidneys have been published (Litterst, 1981; Daley & McBrien, 1982).

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 arised from the administration of the parent conpound. Such repair products could be expected releasing 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 administation may be dependent on the occurrence of ligand substitution processes in which one or more of the ammonia groups bonded to the platinum are replaced by groups such as sulfur atoms that are present in the sulfhydryl groups of proteins. An alternative explanation is that the metabolism of the coordinated guanosine may be responible for the retention of platinum-containing species in the renal tissue (Rosenberg, 1978; Daley & McBrien, 1982, 1984).

New platinum (II) coordination complex produced and used by in this study have generally been screened for antitumor activity and nephrotoxicity using MKN-45 human gastric adenocarcinoma cell-lines, and normal human and rabbit kidney cells, respectively. New synthetic platinum (II) coordination complex, PC exhibited significant *in vitro* antitumor activity aganist human gastric adenocarcinoma cell lines.

A criteria for *in vitro* antitumor activity is generally expressed in cytotoxicity index and is accepted as possible antitumor drugs. PC showed comparable antitumor activity to cisplatin. The comparison of antitumor activities between PC and cisplatn exhibited similar levels in MKN-45 cell-lines. Since the difference of chemical structure between PC and cisplatin is only "DACH-isomer", it can be explained that dissociation time or rate is different after absorbing the compound in the cells due to the different leaving groups.

In this study, PC was less cytotoxic than cisplatin in renal tissues. This new compound is conceivable that modification of the carrier ligand as a DACH and leaving group as a DPPE derived from cisplatin significantly decreased nephrotoxicities. Mortine & Borch (1988) reported the LLC-PK_1 (pig proximal tubular epithelial cell-line) was a good model to evaluate nephrotoxicity induced by cisplatin *in vitro*. In our present study using primary cultured cells showed reliable data instead of LLC-PK_1 cell-line.

In vivo, the apperance of glucose in urine is one of the early signs of proximal tubular dysfunction and therefore we choose 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 for glucose consumption test provided a good association for cisplatin toxicities. 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 than cisplatin, particulary at high concentrations. As mentioned above, however, this new complex has very low nephrotoxicity. Therefore, this new complex may possibly be useful clinically 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

Appleton TG, Bennett MA, Tomkins IB. Effect of chelatering size on spectroscopic and chemical properties of methylplatinum (II) complexes of the ditertiary phosphines $Ph_2P[CH_2]nPPh_2$ (n=1, 2 or 3). *JCS Dalton* 439−446, 1976

Chang SG, Slocum HK, Toth K, Hoffman RM. Glucose consumption end point in primary histoculture indicates recovery of human tumors from drug treatment. *In Vitro Cell Dev Biol* 28A: 585−587, 1992

Chang SG, Lee JH, Hong DH, Lee HL, Chai SE, Hoffman RM. Comparison of glucose-consumption and thymidine-incorporation endpoints in histocultured human superficial bladder 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, Sivingston 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
Connors 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
Cvitkovid E, Spaulding J, Bethune V. Improvement of cis-diamminedichloroplati-num (NSC-119875): Therapeutic index and animal model. *Cancer* 39: 1357−1362, 1977
Daley-Yates PT, McBrien DCH. The inhibition of renal ATPase by cisplatin and some biotransformation products. *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: 3063−3069, 1984
Dobyan DC, Levi J, Jacobs C. Mechanism of cisplatinum nephrotoxicity. *J Pharmacol Exp Ther* 213(3): 551−556, 1980
Einhorm LH, Donohue J. Cis-dismminedichloroplatinum, vinblastine and bleomycin combination chemotherapy in disseminated testicular cancer. *Ann Intern Med* 87: 293−298, 1977
Ettinger LT, Gaynon PS, Krailo MD. A phase II study of carboplatin in children with recurrent or progressive solid tumors. A report from the childrens group. *Cancer* 73: 1297−1303, 1994
Fields KK, Zorsky PE, Hiemenz JW. Ifosfamide carboplatin and etoposide: a new regimen with a broad spectrum of activity. *J Clin Oncol* 12: 544−550, 1994
Gale GR, Walker EM, Atkins LM. Antileukemic properties of dichloro (1,2-diaminocyclohexane) platinum (II). *Res Commun Chem Pathol Pharmacol* 7: 529−533, 1974
Glover D, Glick JH, Weiler C. Phase I/II trials of WR-2721 and cis-platinum. *Int J Radiat Oncol Biol Phys* 12(8): 1509−1512, 1986
Gonzales-Vitale JC, Hayes DM, Sternberg SS. The renal pathology in clinical trials of cis-platinum. *Cancer* 39: 1362−1371, 1977
Gottlieb JA, Drewinko B. Review of the current clinical status of platinum coordination complexes in cancer chemotherapy. *Cancer Chemother Rep* 59: 621−627, 1975
Hamilton TC, Winker MA, Louie KG, Batist G, Behrens BC, Tsuruo T. Augmentation of adriamycin, melphalan, and cisplatin cytotoxicity in drug-resistant and sensitive human ovarian carcinoma cell lines by buthionine sulfoximine mediated glutathione depletion. *Biochem Pharmacol* 34: 2583−2586, 1985
Hardaker WT, Stone RA, McCoy R. Platinum toxicity. *Cancer* 34: 1030−1035, 1974
Harstrick A, Casper H, Guba R, Wilke H, Poliwoda H, Schmoll HJ. Comparison of the antitumor activity of cisplatin, carboplatin and iproplatin against established human testicular cancer cell lines *in vivo* and *in vitro*. *Cancer (Philadelpia)* 63: 1079−1083, 1989
Hill JM, Loeb E, MacLellan A. Clinical studies of platinum coordination compounds in the treatment of various malignant disease. *Cancer Chemother Rep* 59: 647−652, 1975
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 nephrotoxicity. *Arch Toxicol* 59(3): 167−171, 1986
Jones MM, Beaty JA. Rate of displacement of guanosine by cyanide from cis-diamminebis (guanosine) platinum (II) chloride determined by using [^{13}C]-NMR spectroscopy. *Inorg Chem* 30: 1584−1592, 1991
Jung JC, Kim YK, Park SJ, Chung JH, Chang SG, Lee KT, Beak 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, 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
Jung JC, Lee MH, Chang SG, Rho YS. Antitumor activity and nephrotoxicity of the novel platinum (II) complex. *Korean J Pharmacol* 31: 1, 103−114, 1995
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, 1979
Litterst CL. Alterations in the toxicity of cis-dichlorodiammineplatinum (II) and in tissue localization of platinum as a function of NaCl concentration in the vehicle of administration. *Toxicol Appl Pharmacol* 61: 99−104, 1981
Meijer S, Mulder NH, Sleijfer DT, Schraffordt KH. Nephrotoxicity of cisplatinum during remission-induction and maintenance chemotherapy of testicular car-

cinoma. *Cancer Chemother Pharmacol* 8(1): 27−30, 1982

Mortne TJ, Borch RF. Quiescent LLC-PK_1 cells as a model for cis-diaminedichloro 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 Immunol Methods* 65: 55−61, 1973

Ridgway HJ, Speer RJ, Hall JM. Analogs of sulfato 1,2-diaminocyclohexane platinum (II): Modifications in leaving 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, Grimley EB, Thomson AJ. The inhibition of growth or cell division in Escherichia coli by different 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