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[Pt(II) (cis-DACH) (DPPE)] · $2NO_3$: A Novel Class of Platinum Complex Exhibiting Selective Cytotoxicity to Human Ovarian Carcinoma Cell Lines and Normal Kidney Cells

Jee-Chang JUNG[1]*, Min-Ho CHU[1], Sung-Goo CHANG[2], Kyung-Tae, LEE[3] and Young-Soo RHO[3]

[1]Department of Pharmacology & [2]Urology, School of Medicine, [3]College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea

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Abstract – Cisplatin, a platinum-complex, is currently one of the most effective compounds used in the treatment of solid tumors. However, its use is limited by severe side effects such as renal toxicity. Our platinum-based drug discovery program is aimed at developing drugs capable of diminishing toxicity and improving selective cytotoxicity. We synthesized new Pt (II) complex analogue containing 1,2-diaminocyclohexane (DACH) as carrier ligand and 1,2-bis (diphenylphosphino) ethane (DPPE) as a leaving group. Furthermore, nitrate was added to improve the solubility. A new series of [Pt(cis-DACH)(DPPE)] · $2NO_3$ (PC) was synthesized and characterized by their elemental analysis and by various spectroscopic techniques [infrared (IR), ^{13}carbon nuclear magnetic resonance (NMR)]. PC demonstrated acceptable and significant antitumor activity against SKOV-3 and OVCAR-3 human ovarian carcinoma cell lines as compared with that of cisplatin. The cytotoxicity of PC in normal cells was found quite less than that of cisplatin using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), [^{3}H]thymidine uptake and glucose consumption tests in rabbit renal proximal tubular cells, human renal cortical cells and tissues. In conclusion, PC is considered to be more selective cytotoxicity toward human ovarian cancer cells than normal human/rabbit kidney cells.

Keywords□ cytotoxicity, platinum compound, renal proximal tubule, renal cortical cells.

Cisplatin (cis-diamminedichloroplatinum (II)) is one of the first-line chemotherapeutic agents for the treatment of ovarian carcinoma, testicular cancer and cancer of the head and neck, bladder cancer (Lippman *et al.*, 1973). The platinum 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 inhibitory effects on bacterial replication were subsequently compounds in the presence of chloride and ammonium ions. The antineoplastic activity of cisplatin is attributed to its preferential reaction with N-7 atoms on the guanine bases in DNA; such reaction ultimately from compounds in which both chlorides are replaced by nucleic acid groups (Sherman and Lipard, 1987). However, its usefulness is compromised by its propencity to cause several dose-limiting toxicities, including nephrotoxicity, nausea, vomiting, neurotoxicity, ototoxicity, and myelosuppression (Harrap *et al.*, 1980; Ward and Fauvie 1976), and its potential to induce resistance in responsive tumor types (Graeff *et al.*, 1988). Nephrotoxicity is the observations that for platinum complex (Litterst *et al.*, 1977).

The prominent contribution thus far, in over 20 years of study, has been the discovery of the 1,2-diaminocyclohexane (DACH) as a carrier ligand (Burchenal *et al.*, 1979). These DACH complexes retain activity in cisplatin-acquried resistant murine leukemias, both *in vitro* DACH complexes [e.g., 1,2-diaminocyclohexane (4-carboxyphthalato) platinum (II) and [1,1-diaminomethycyclohexane sulfato platinum (II)] have entered Phase I/II clinical trial but have sequently been dropped, primarily due to unacceptable toxicity (Canetta et al., 1990; Noji *et al.*, 1981).

Consequently, there is a great interest in obtaining agents that have less toxicity and have more favorable therapeutic indices. To accomplish this task, we synthesized a new platinum analogue containing DACH as a car-

*To whom correspondence should be addressed.

rier ligand and 1,2-bis (diphenylphosphino) ethane (DPPE) as a leaving group.

The present study reports on the synthesis of new platinum (II) coordination complex and its cytotoxicities were evaluated with SKOV-3 and OVCAR-3 human ovarian adenocarcinoma cell lines, rabbit renal proximal tubular cells, cultured human renal cortical cells and cortical tissues as a compared with those of cisplatin.

MATERIALS AND METHODS

Materials

1,2-Bis (diphenylphosphino) ethane (DPPE) and cis-1, 2-diaminocyclohexane (DACH) were obtained from the Tokyo Chemicals. Hormones, transferrin, and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A). Cell culture supplies (powdered medium EDTA/trypsin and soybean trypsin inhibitor) were from Life Technologies (Grand Island, NY, U.S.A). Class IV collagenase was obtained from Worthington (Freehold, NY, U.S.A). Iron oxide was prepared by the method of Cook and Pickering (1958). Stock solutions of iron oxide in 0.9% NaCl were sterilized using an autoclave and diluted with phosphate buffered saline (PBS) before use.

Methods

Synthesis of platinum(II) complex

(1) (cis-1,2-diaminocyclohexane) dichloroplatinum (II)-[Pt(cis-DACH) Cl_2]: To a solution of K_2PtCl_4 (2.50 g, 6.02 mM, in H_2O (80 ml) was added a solution of cis-DACH. 2HCl (1.13 g, 6.02 mM) in water (20 ml). The mixture was adjusted to pH 6.5 by titration with 5% NaOH and stirred for 30 min at room temperature. The yellow crystals were formed and filtered. The yellow crystals were dried in vacuum evaporation; The final yield was 1.84 g.

(2) (cis-1,2-diaminocyclohexane) dinitrate platinum (II)-[Pt(cis-DACH)-$(NO_3)_2$]: To a suspension of Pt (cis-DACH) Cl_2 1 g (6.02 mM) was treated stepwise a solution of $AgNO_3$ (890 mg, 5.26 mM) in distilled water (10 ml). The reaction mixture was stirred for 24 hrs at room temperature. The reaction product of AgCl was filtered off. The filtrate was concentrated under reduced pressure and dried with lyophillization; The final yield was 810 mg.

(3) {1,2-Bis (diphenylphosphino)ethane} (cis-1,2-diaminocyclohexane) Pt (II) nitrate-[Pt (cis-DACH) (DPPE)] $(NO_3)_2 \cdot H_2O$: To a solution of Pt (cis-DACH)-$(NO_3)_2$ (500 mg, 1.15 mM) in 15 ml of H_2O was added a solution of DPPE (460 mg, 1.15 mM) in 20 ml of acetone. The mixture was stand for 1 hr and evaporated under reduced pressure. The yellow crystals were formed and dried with lysophilization. The product was recrystalized from H_2O; The final yield was 428 mg.

Cell culture

Ovarian adenocarcinoma cell cultures

SKOV-3 and OVCAR-3 human ovarian adenocarcinoma cells were obtained from Korean Cell Line Bank, Seoul National University (Seoul, Korea). The basal medium RPMI 1640 containing 20 mM sodium bicarbonate, 15 mM HEPES, 92 IU/ml penicillin and 200 μg/ml streptomycin. RPMI 1640 supplemented with 10% fetal bovine serum (FBS) was utilized as the growth medium for SKOV-3 and OVCAR-3 cells. Stock cultures of SKOV-3/ OVCAR-3 cells were maintained in RPMI medium in a humidified 5% CO_2/95% air environment at 37℃.

Primary rabbit renal proximal tubular cell cultures

Primary rabbit renal proximal tubular cell cultures were prepared by a modification of the method of Chung *et al.* (1982) and Jung *et al* (1992). To summarize, the kidneys of a male New Zealand white rabbit (2 to 2.5 kg) were perfused via the renal artery, first with PBS, and subsequently with DME/F12 containing 0.5% iron oxide (wt/vol), such that the kidney was turned gray-black in color. Renal cortical slices were homogenized with 4 strokes of a sterile Dounce homogenizer (type A pestle Bellco, U.S.A), and the homogenate was poured first through a 253 and then a 83 mesh filter. Tubules and gomeruli on top of the 83 filter were transferred into sterile serum-free modified DME/F12 medium containing a magnetic stir bar. Glomeruli (containing iron oxide) were removed with the 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 transfered into tissue culture dishes. Medium was changed one day after plating and every two days thereafter.

Primary human renal cortical cell culture

Normal kidney tissue was freshly excised from patient undergoing radical nephrectomy in patient with renal cell carcinoma. Renal cortical tissues were washed 3 or 4 times with DME/F12 (1:1) medium supplemented with penicillin/streptomycin. A single-cell suspension was obtained by mechanical disaggregation with sterilized surgical knife and subsequent incubation with collagenase (0.124 mg/ml) and trypsin inhibitor (2.5 mg/ml) for 2 min. The process was stopped by centrifugation (1000

rpm for 5 min) and the particles of kidney cortical tissue was suspensed with DME/F12 medium supplemented with insulin (0.5 μg/ml), transferrin (5 μg/ml), hydrocortisone (5×10^{-8} M), triiodothyronine (5 μg/ml), prostaglandin E1 (5×10^{-8} M) and FBS (1%). This suspensed medium was seeded on culture dish in an incubator at 37℃ maintaining highly humidified atmosphere 5% CO_2/95% air. After 2 weeks incubation, the cells were confluent and used for experiments.

Cultured human renal cortical tissues

Normal human kidney tissue, identified by frozen section at the time of radical nephrectomy, was transported in a sterile 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 top of previously hydrated Spongostan gel (1×1 cm) (Health Design Indust. Rochester, N. Y, U.S.A). One gel was put in each well of six-well plate. Three mililiters of Eagle's minimal essential medium (MEM) supplemented with 10% FBS and 50 μg/ml gentamicin and cefotaxime at a final concentration of 1 μg/ml were added to each well. 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℃. The cultures underwent sterile media changes every 3 day. Culture was continued up to 3 weeks after explantation. Specimens were exposed to media containing newly formed platinum complex and cisplatin for 3 days. After drug treatment, the specimens were washed with PBS and fresh media (Freeman and Hoffman, 1986; Chang *et al*, 1992).

Antitumor activity

Human ovarian adenocarcinoma cell-lines SKOV-3 and OVCAR-3 were cultured in 20 ml of RPMI medium supplemented with 100 μg/ml streptomycin/ penicillin and 10% fetal calf serum (FCS) in incubators maintaining highly humidified 5% CO_2/95% air at 37℃. After 3 days culture, all cell lines were dissociated with trypsin-EDTA for dispersal and centrifuged 1,000 rpm for 5 min. The pellets were suspended with fresh medium. MTT assay was done as described in nephrotoxicity.

Nephrotoxicity

MTT assay

This was performed essentially described as previously (Denizot and Lang, 1986). Briefly, the confluent primary rabbit renal proximal tubular and human renal cortical cells were disaggregated using 0.02% EDTA in 0.05% trypsin. Single cell suspension were then produced by centrifugation (1000 rpm, 10 min), resuspending in DME/F12 medium (10^6 cells/ml). This suspension was seeded 10^5 cells per well in 96-well plate in 100 μl of medium. Drugs were added at various concentration (final concentration; 5, 15, 50, 150 and 300 μM) and cultures were incubated for 48 hrs in an incubator maintaining highly humidified atmosphere of 5% CO_2/95% air at 37℃. The 50 μl of medium containing MTT (5 mg/ml) was added to each well. After 4 hrs of exposure, the medium was removed and washed with PBS, and then 50 μl of DMSO was added to each plate to solublize the precipitates. The plate was transferred to a 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 test agents.

Thymidine uptake test

Cultured primary rabbit renal proximal tubule cells and human renal cortical cells were seeded at 10^6 cells per well in 24 well plates. After 1 hr incubation drug were added for 48 hrs under humidified incubator 5% CO_2/95% air at 37℃. Thereafter, [^{3}H]-thymidine (1 μCi/ml; specific radioactivity) was then added to each well, and cells were again incubated for 24 hrs in the same humidified incubator. After trypsin-EDTA treatment, all cells were collected and washed 2 times with 10% TCA and phosphate buffer. The cells were then solublized with 0.5 M-NaOH for 2 hrs at 37℃. The amount of radioactivity was determined by neutralizing with 0.5 M HCl, adding scintillating cocktail (Scint-AXF, Packard, C.T, U.S.A) and counting in a β-counter (Beckman LS 5000TD, U.S.A).

Glucose consumption test

50 μl of culture medium were taken every 24 hr for determination of medium glucose content in triplicate using the HK 20 assay kit from Sigma (St. Louis, M. O, U. S.A). Measurements were made by monitoring the changes in optical density at 340 nm due to the reduction of NAD catalized by hexokinase with the glucose substrate before and after cisplatin and PC treatment.

The glucose content of the medium as plotted as a semilog plot versus time after medium renewal using the Sigma plot program (Jandel Scientific, Corte Madera, CA, U.S.A).

A simple exponential model of glucose consumption was then fitted to the data with the Systat program

(Systat Inc. Evanston, IL, U.S.A). 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 of the natural log of 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 3-day measurement period (one period).

RESULTS

Pt(II) Complex Synthesis

Synthetic $PtCl_2$ (cis-DACH) is a yellow crystal and water insoluble. Water soluble Pt-dinitrate (cis-DACH) is prepared by replacement of Cl with nitrate. Final products of [Pt(cis-DACH) (DPPE)]. $2NO_3$(PC) was synthesized by mixing 1:1 ratio of DPPE to above prepared compound.

The platinum complex was submitted for elemental analysis prior to biological evaluation. Analytical data is presented (Table I). There is no significant difference betwen PC in IR spectrum and the functional bands of this compound shown in Table II. ^{13}C-NMR chemical shift and coupling constants are exhibited in Table III.

Table I. Result of elemental analysis of platinum (II) complex

Compound	Calculated (%)			Found (%)		
	H	C	N	H	C	N
PC	4.88	44.30	6.46	4.90	45.00	6.50

PC: [Pt (cis-DACH) (DPPE)] $(NO_3)_2$.

Table II. IR spectra of DPPE and their mixed ligand platinum (II) complex

Compounds	νNH	νCH (Phenyl)	δNH	νP-C (Phenyl)	νNO3 (cm^{-1})
DPPE		3067 (W)		1432	
PC-2	3450 3192	3053 (W)	1592	(VS)	1382 819

PC: [Pt (cis-DACH) (DPPE)] $(NO_3)_2$.

Antitumor Activity

The *in vitro* cytotoxicity of a new antitumor platinum complex, PC, was evaluated against human ovarian carcinoma cell lines along with those of cisplatin using a MTT assay.

Fig. 1 shows the result obtained after exposure of 5, 15, 50, 150 and 300 μM against SKOV-3 human ovarian adenocarcinoma cell-line. PC showed concentration-dependent increase in antitumor activity (cytotoxicity index, CI: 61.9% for 150 μM) and as active as that of cisplatin at 300 μM.

Fig. 2 shows the results obtained when these drugs were exposured to OVCAR-3 human ovarian carcinoma cell line. Antitumor activity of this new Pt(II)-complex against OVCAR-3 cells is also dependent on concentration and quite comparable to that of cisplatin.

As showin in Fig. 1 and Fig. 2, PC showed dose dependant increase *in vitro* cytotoxic activity against ovarian human ovarian carcinoma cell lines and this antitumor activity of PC aganist above these two cell lines is quite comparable to that of cisplatin.

Nephrotoxicity

To examine whether new Pt (II) complex, PC, was accompanied nephrotoxicity, we tested several kidney cells

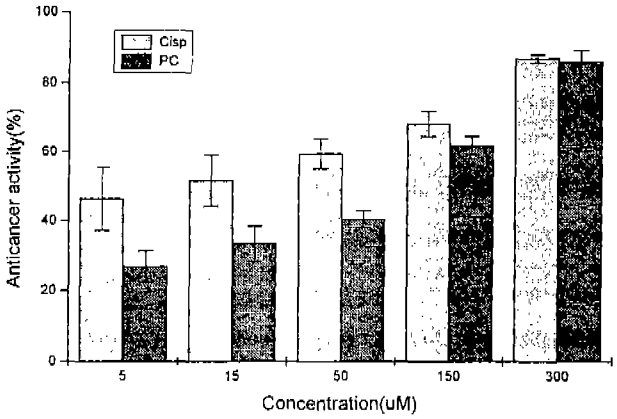

Fig. 1. Anticancer activities of platinum (II) complexes on the SKOV-3 human ovarian adenocarcinoma cell-line. Cisp; Cisplatin. PC; [Pt(II) (cis-DACH) (DPPE)] · $2NO_3$.

Table III. ^{13}C-NMR spectra of DPPE and Pt (II) complex

Compounds	Phenyl group				Bridging	CH	Diamine moiety			Solvent
	δC_1 (J P-C)	$\delta C_{2,6}$ (J P-C)	$\delta C_{3,5}$ (J P-C)	δC_4	δC_7	δC_8	$\delta C_{1,2}$	$\delta C_{3,6}$	$\delta C_{4,5}$	
DPPE	139.2 (t,7.5)	133.8 (t,8.8)	129.5 (t,3.5)	130.5 (s)	25.1 (s)					CD_2Cl_2
[Pt (cis-DACH)	a)	134.6 (t,7.0)	129.4 (t,6.4)	133.1 (s)	24.3 (s)		60.8 (s)	31.6 (s)	24.1 (s)	CMSO
(DPPE)] $(NO_3)_2$		132.2 (t,8.6)	126.3 (t,4.0)	132.4 (s)						

a): Resonance not observed. b): ppm from TMS.

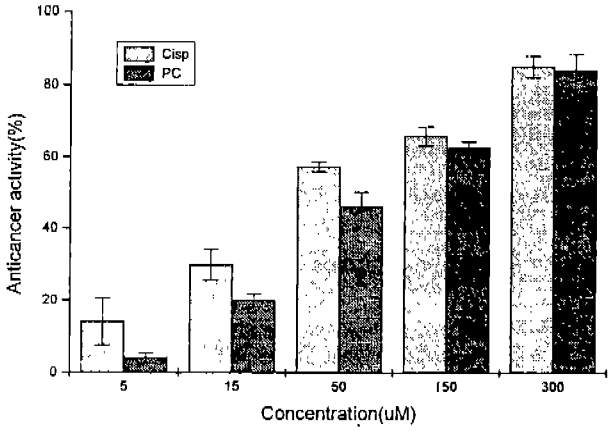

Fig. 2. Anticancer activities of platinum (II) complexes on the OVCAR-3 human ovarian adenocarcinoma cell-line. Cisp; Cisplatin. PC: [Pt(II) (cis-DACH) (DPPE)] · $2NO_3$.

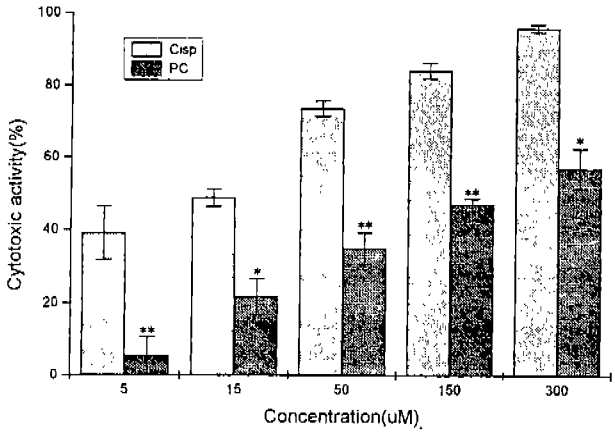

Fig. 3. Cytotoxic activities of platinum (II) complexes on the proximal tubule cells of the normal rabbit kidney. Cisp; Cisplatin. PC; [Pt(II) (cis-DACH)(DPPE)] · $2NO_3$. *Significantly different from cisplatin-control (*P<0.05, **P<0.01).

and tissue such as rabbit proximal tubule cells and human cortical cells and tissues with several cytotoxicity assays.

Rabbit renal proximal tubular cells

The cytotoxicities of cisplatin and PC against primary rabbit renal proximal tubular cells as determination by MTT assay are shown in Fig 3. PC (CI: 5.4%) showed less cytotoxic at 5 μM as compared with that of cisplatin (CI: 39.2%). At a concentration of 50 and 300 μM, PC showed around 2-fold less cytotoxic than that of cisplatin.

In addition to MTT assay, cytotoxicities were determined using [^{3}H]-thymidine uptake assay. Results using this assay are shown in Table IV. PC showed 59.2% of [^{3}H]-thymidine uptake as compared with that of cisplatin (1.5%) at 500 μM. These result indicates that cytotoxicity of PC was significantly less than that of cisplatin,

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

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

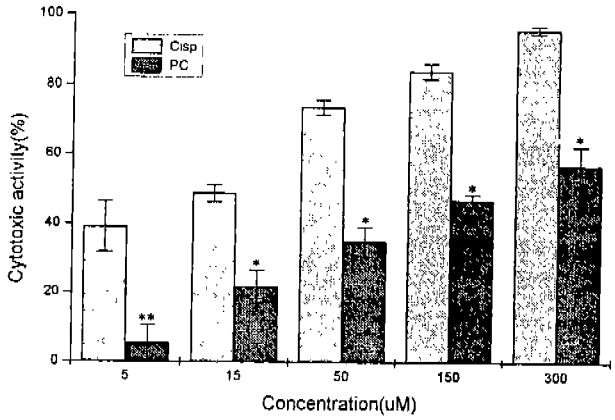

Fig. 4. Cytotoxic activities of platinum (II) complexes on the renal cortical cells of human kidney. Cisp; Cisplatin. PC; [Pt (II) (cis-DACH) (DPPE)] · $2NO_3$. *Significantly different from cisplatin-control (*P<0.05, **P<0.01).

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

Group	^{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 Pt (II)-complexes in culture medium: 5×10^{-5} M, PC: [Pt (cis-DACH) (DPPE)]$(NO_3)_2$, Values are means± S.E. All the incorporation was determined in triplicate.

and [^{3}H]-thymidine uptake assay is more sensitive than MTT test (up to 7-fold).

Human renal cortical cells

PC showed significantlyless cytotoxicity (CI: 10.0%) than that of cisplatin (CI: 70.2%) at 50 μM (Fig. 4) and does dependent cytotoxicity feature against human kidney cortical cells. Table V shows that [^{3}H]-thymidine incorporation is significantly inhibited by cisplatin (1.4%) as compared with that of PC (42.3%).

Human renal cortical tissues

In glucose consumption, one period is defined as more

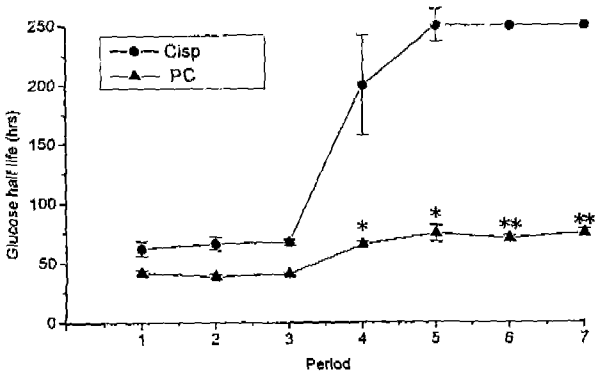

Fig. 5. Nephrotoxicity on 3 weeks histocultured human kidney. Toxicity was measured by glucose consumption. Each drug exposed for 72 hrs with to 10 μM in concentration. Cisp; Cisplatin. PC; [*Pt (II) (cis-DACH) (DPPE)*] · $2NO_3$. *Significantly different from cisplatin-control (*P<0.05, **P<0.01).

than 3 times mesurement per day in 4 week-culture of human renal cortical tissue. The half-life of glucose before adding of PC is approximately 28~39.6 hrs and does not show any statistical significance. However, half-life of glucose was significanty increased at 4 period, and then showed more than 190 hrs at 5, 6 periods. However, the effect of PC was less marked than cisplatin (Fig. 5).

DISCUSSION

Since Rosenberg *et al.* (1965, 1967, 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 use is often complicated 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 platinum containing compounds which have more favorable therapeutic indices and circumvent resistance.

The structure-activity relationship is clarified by the effect of carrier ligands and leaving groups *in vivo* antitumor activity. 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 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; Gale *et al.*, 1974; Ridgway *et al.*, 1977). Several DACH compounds exist such as cis-DACH, trans-*l*-DACH and trans-*d*-DACH. Among these DACH derivatives, trans-*l*-DACH has been known to have significant antitumor activity (Inagaki and Kitani, 1986). Moreover, it is essential to consider the leaving group which is important factor to influence the activity of Pt-complexes. The Pt (II)-complexes appear to peneterate cell membrane by diffusion and the leaving group is displaced directly by hydrolysis. This is responsible for formation of the activated species of drug, which reacts with the DNA (i.e., with the guanine N7 forms), resulting in inhibition of DNA replication and cytotoxic effect (Tashiro, 1988). In addition to its reactive with DNA, Pt (II)-complexes can react with protein-bound sulfhydryl groups of the proximal tubules with resulting significant toxic action on renal function (Odenheimer and Wolf, 1982).

These studies indicate that the dissociation of leaving group is important factor for antitumoral and toxic activity. However, when the rate of dissociation is much higher, it causes toxicological effects because of reaction with normal protein instead of DNA in cancer cells. Contrastly, when the dissociation rate is too low, it is excreted extracellular compartment before showing any antitumor activity.

The mechanism of nephrotoxicity induced by Pt (II)-complexes is not completely understood. Investigators have demonstrated that cytotoxicity induced by a variety of drugs may be attributable at least in part to inhibition of of blood-flow in kidney or depletion of intracellular glutathione (Meijer *et al.*, 1982; Levi *et al.*, 1980).

Dobyan *et al.*, (1980) have reported site-specific injury to the pars recta (S_3) segement of the proximal tubules. Gonzalez-Vitale *et al* (1977) noted that the distal tubule is the most consistently damaged region in human kidney. Furthermore, a number of investigators (Porter *et al*, 1981; Jones *et al*, 1980) suggested that both of proximal and distal tubules have been damaged.

This nephrotoxicity induced by Pt (II)-complexes has been largely abrogated by the rutine use of hydration and diuresis (mannitol) and sulfnucleophiles (WR-2721 and diethyldithiocarbamate) (Jones *et al.*, 1986; Glover *et al.*, 1986; Bodenner *et al.*, 1986). It is well documented that mannitol reduce cisplatin nephrotoxicity by diluting its tubular urinary concentration rather than by altering its half-life, plasma clearance or total urinary excretion.

New Pt (II)-containing analogue have generally been screened for antitumor activity and nephroroxicity using two cancer cell lines (SKOV-3, OVCAR-3 human ova-

rian adenocarcinoma cells) and human/rabbit kidney cells, respectively. New synthetic Pt(II)-complex, PC, exhibited significant antitumor activity aganist SKOV-3 and OVCAR-3 cell lines.

A criteria for antitumor activity *in vitro* is generally expressed in cytotoxicity index and more than 50% in cytotoxicity index is accepted as positive antitumor drugs. PC showed comparable antitumor activity to cisplatin in SKOV-3 and OVCAR-3. However, PC demonstrated significant antitumor activity as compared with that of cisplatin at low concentration.

The results obtained here presented that PC had less cytotoxic than cisplatin. This is conceivable that modification of the carrier ligand as a DACH and leaving group as a DPPE derived from cisplatin significantly changed antitumor activity and nephrotoxicity.

Mortine and Borch (1988) reported that LLC-PK_1 (pig proximal tubular epithelial cell-line) is a good model to evaluate nephrotoxicity induced by cisplatin *in vitro*. These studies using primary cultured kidney cells showed reliable data instead of LLC-PK_1 cell-line.

In vivo, the appearance of glucose in urine is one of the early signs of proximal tubular dysfunction and therefore we choose glucose consumption as a parameter to assess the nephrotoxicity in human renal cortical tissue.

These results is reliable as that of renal cortex because human renal cortical tissue is maintained with collagen gel through three dimensional culture method (Freman and Hoffman, 1986; Chang *et al.*, 1992).

Further development of these rabbit renal proximal tubular cells and human renal cortical cell culture system may have value in detecting potential nephrotoxicity and in studying their mechanism.

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