Korean J. of Pharmacology
Vol. 31, No. 1, pp. 103-114, 1995

Antitumor Activity and Nephrotoxicity of the Novel Platinum(II) Coordination Complex

Jee-Chang Jung[1], Moon-Ho Lee, Sung-Goo Chang* and Young-Soo Rho**

Department of Pharmacology and Urology, College of Medicine, Department of Medicinal Chemistry**, College of Pharmacy, Kyung Hee University, Seoul 130-701, Korea*

ABSTRACT

Platinum coordination complexes are currently one of the most 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 antitumor activity. 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(trans-d-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 antitumor activity aganist P388, L-1210 lymphocytic leukemia cells and SK-OV3 human ovarian adenocarcinoma cells, and significant activity as compared with that cisplatin. The toxicity of PC was found quite less than that of cisplatin using MTT, [^{3}H] thymidine uptake and glucose consumption tests in rabbit proximal tubule cells, human kidney cortical cells and human renal cortical tissues. Based on these results, this novel platinum compound represent a valuable lead in the development of a new anticancer chemotherapeutic agent capable of improving antitumor activity and low toxicity.

Key Words: Platinum coordination complex, Antitumor activity, Nephrotoxicity, Glucose consumption tests

INTRODUCTION

The platinum coordiantion 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 shown to be due to the formation of inorganic platinum-containing compounds in the presence of chloride and ammonium ions. Cis-diamminedichloroplatinum (II) (cisplatin) was found to be the most active of theses ubstance in experimental tumor system using sarcoma 180 (Connor, 1974; Kociba *et al.*, 1973). The introduction of cisplatin into the reponse rate of some tumor types (ovarian cancer, cancer of head and neck, bladder cancer), notably testicular carcinoma. However, its usefulness of cisplatin has been limited by its propensity to cause several dose-limiting toxicities, including nephrotoxicity, oto-

[1]To whom correspondence should be addressed.

This study was supported by grant from Kyung Hee University(1994).

toxicity, myelosuppression and its potential to induce resistance in responsive tumor types (Ward and Fauvie, 1976; Ward and Young, 1976; Kociba *et al.*, 1971; Schaeppi *et al.*, 1973). The major dose-limiting side effect of cisplatin is nephrotoxocity (Gottlieb *et al.*, 1975). It is known that renal cortical accumulation of cisplatin lead to necrosis of the proximal tubule and late development of internal cysts (Hardaker *et al.*, 1974; Krakoff. 1979; Jacob *et al.*, 1980; Litterest. 1974).

Cisplatin-induced nephrotoxicity has been largely abrogated by the routine use of hydration, mannitol (Cvitkovic *et al.*, 1977) or diuretics like furosemide (Hill *et al.*, 1975; Einhorn *et al.*, 1977).

Platinum coordiantion complexes are consisted of essential metal of platinum susbstituble-leaving group and unsubstituble carrier ligand. Carrier ligand is responsible for antitumor activity and spectrum. Chemical structure of amine as a carrier ligand is an important factor to influence the antitumor activity. This antineoplastic activity of cisplatin is attributed to its preferential reaction of carrier ligand with the N-7 atoms on the guanidine base in DNA, such reactions ultimately form compounds in which both chlorides are replaced by nucleic acid groups.

Antitumor activity of platinum complexes is also depends on the stereo-activity of carrier ligand because DNA contains stereoselectivity. It has been reported that the 1.2-diaminocyclohexane (dach) carrier ligand shows particular promise because of excellent antitumor activity, low toxicity, and each of cross resistance with cisplatin (Clear *et al.*, 1973; Connors *et al.*, 1972; Gale *et al.*, 1974; Ridgway. 1977).

Leaving group is concerned with the stability, reactivity (Tashiro, 1988) and water-solubility. Pt (II) complexes appear to enter the cells by diffusion. The hydrolysis of leaving group is responsible for formation of the activated species of the drug, which reacts with DNA, resulting in inhibition of DNA replication. This explanation suggests that antitumot activity of Pt (II) complexes is closely correlation with the replaced rate of leaving group in vivo.

To date, there has been a notable investigation for novel platinum chemistry adressing stability, broad antitumor activity, and lower nephrotoxicity. The antitumor activity of platinum complexes containing dach carrier ligand was investigated by Connors (1972), Clear (1973), and Gale (1974). Kidani *et al* (1985) synthesized Pt (oxalato) (trans-1-dach) [1-OHP] and Pt (malonato) (trans-1-dach) [1-PHM] using oxalate/malonate with selected trans-1-dach among trans-1. trans-d and cis-isomers.

Our platinum-based drug discovery program is aimed at developing drugs capable of broadening the antitumor activity and decreasing side effect. To assist in these objective we have recently synthesized a new compound; [Pt (II) (trans-d-dach) (DPPE)]. $(NO_3)_2$.

The present study reports on the synthesis of new platinum (II) coordination complex and their antitumor activity and nephrotoxicity were evaluated with cancer cell lines, rabbit proximal tubule cells, human kidney cortical cells and histocultured human renal cortical tissues as a compared with those of cisplatin.

MATERIALS AND METHODS

Materials

Platinum agents; 1,2-Bis(diphenylphosphino) ethane (DPPE) and 1,3-bis (diphenyl phosphino) propane (DPPP) were obtained from the Tokyo chemicals (JAPAN). Trans-d-1,2-diaminocyclohexane was kindly supplied by Dr. Kidani Department of Pharmacy, University of Nagoya (JAPAN). This trans-d-dach was seperated from trans-dl-dach purchased from Aldrich Chemical Co. (Milwaukee, WI, USA)

The chemical structure of platinum analogue were presented:

PC [Pt (trans-d-dach) (DPPE)].$2NO_3$

Hormones, transferrin, and other chemicals were purchased from Sigma Chemical Corp. (St. Louis, MO, USA). Powdered medium and soybean trypsin inhibitor were from Life Technologies (Grand Island, NY, USA). Class IV collagenase was from Worthington (Freehold, NY, USA).

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 PBS prior to use.

Methods

1) Synthesis of platinum (II) complexes

(1) (trans-d-1,2-diaminocyclohexane) dichloroplatinum (II)-[Pt (trans-d-dach)Cl_2]: To a solution of K_2PtCl_4 (420 mg, 1.02 mM) in H_2O (30 ml) was added a solution of trans-d-dach. 2HCl (190 mg, 1.01 mM) in water (15 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 470 mg.

(2) (trans-d-1,2-diaminocyclohexane) dinitrate platinum (II)-[Pt(trans-d-dach). $(NO_3)_2$]: To a suspension of Pt (trans-d-dach) Cl_2 380 mg(1 mM) was treated stepwise a solution of $AgNO_3$(340 mg, 1 mM) in distilled water (10 ml). The reaction mixture was stirred for 24h at room temperature. The reaction product of AgCl was filtered off. The filtrate was concentrated under reduced pressure and dried with lysophillization; The final yield was 310 mg.

(3) {1,2-Bis (diphenylphosphino)ethane} (trans-d-1,2-diaminocyclohexane) Pt(II) nitrate-[Pt(trans-d-dach)(DPPE)]$(NO_3)_2.H_2O$: To a solution of Pt (trans-d-dach)$(NO_3)_2$ (433 mg, 1 mM) in 10 ml of H_2O was added a solution of DPPE (400 mg, 1 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 375 mg.

2) Cell culture

Cell culture environment

Kidney cell cultures were maintained in a humidified 5% CO_2/95% air mixture at 37℃. The basal culture medium, a 50:50 mixture of Dulbecco's modified Eagle's medium and Ham's F12(DME/F12) medium was supplemented with 15 mM HEPES buffer, 1.2 μg/ml sodium bicarbonated, 192 IU/ml penicillin, and 200 μg/ml streptomycin. Water for medium preparation was treated with a Millipore Reverse Osmosis System, followed by treatment with a MIlli-Q reagent grade water system. The Milli-Q system had a carbon cartridge, two mixed bed resins, and an ultrafiltration cartridge (Millipore Corp., Bedford, MA USA). Growth supplements were added to the serum-free basal medium immediately before their use for tissue culture. Primary rabbit kidney proximal tubule cell cultures were cultured in serum-free basal medium supplemented with bovine insulin (5 μg/ml), human transferrin (5 μg/ml), and hydrocortisone (5×10^{-8}M). This medium, medium RK-1, was first described by Chung *et al.* (1982)

(1)Primary rabbit kidney proximal tubule cell culture: Primary rabbit kidney proximal tubule cell cultures were prepared by a modification of the method of Chung *et al.* (182) 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 phosphate buffered saline (PBS), and subsequently with DME/F12 containing 0.5% iron oxide (wt/vol), such that the kidney was turned grey-black in color. Renal cortical slices were homogenized with 4 strokes of a sterile Dounce homogenizer(type A pestle Bellco, USA), and the homogenate was poured first through a 253 and then a 83 mesh filter. Tubules and gomerugli on top of the 83 filter were transferred into sterlie 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 brielfy 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.

(2) Primary human kidney cortical cell culture: Normal kidney tissue was freshly excised from patient undergoing abdominal operation. Kidney cortical tissues were washed 3 or 4 times with DMF/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 suspened 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 E_1(5×10^{-8}M) and fetal bovine serum (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 (Jung *et al.* (1992)).

3) Histoculture

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, NY, USA). One gel was put in each well of six-well dishes three mililiters of Eagle's minimal essential medium (MEM) (GIBCO, USA) supplemented with 10% fetal bovine serum (GIBCO, USA) 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 days. Histoculture 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 phosphate-buffered saline and fresh media.(Freeman and Hoffman, 1986; Chang *et al.*, 1992).

4) Antitumor activity

Mouse leukemia cancer cell line L-1210, P-388 and human ovarian adenocarcinoma cell line (SKOV-3) were cultured in 20 ml of RPMI medium supplemented with 10 μg/ml streptomycin/penicillin and 10% fetal calf serum (FCS) in incubators maintaining highly humidified 5%CO_2/95% iar 37℃. M-14 melanoma cells were cultured under the same condition above explained except DMEM medium. After 3 days culture, all cell lines were dissociate with trypsin-EDTA for dispersal and centrifuged 1,000 rpm for 5 min. The pellets were suspended with fresh medium.

Individual wells of 96-well tissue culture microtiter plate were inoculated with 0.1 ml of the appropriate media containing 10^5 cells. Cisplatin and novel pt(II)-complex were added at various concentrations. After 48 hr incubation, 0.05 ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (5 mg/ml) were added to each plate and incubated]for 4 hr. Thereafter, 0.05 ml of DMSO were added and absorption were read at 630 nm and automatically recorded with Elisa. The control compound is cisplatin.

5) Nephrotoxicity

(1) MTT assay: This was performed essentially described as previously (Bosanquet *et al.*, 1983). Briefly, the confluent primary rabbit kidney proximal tubules and human kidney cortex 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 (10^6cells/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, 50 and 500 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 hr of exposure, the medium was removed and washed with PBS, and then 50 μl of DMSO was added to each plate to solubilize 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.

(2) Thymidine uptake test: Cultured primary rabbit kidney proximal tubule cells and human kidney cortical cells were seeded at 10^6 cells per well in 24 well plate. After 1 hr incubation drug were added for 48 hr 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 hr 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 0.5M-NaOH for 2 hr at 37℃. The amount of radioactivity present was determined by neutralizing with 0.5 M HCl, adding scicillating cocktail (Scint-AXF, Packard, CT) and counting in a β-counter (Beckman LS 5000TD).

(3) 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 it from Sigma (St. Louis).

Measurement were made by monitoring the

changes in optical density at 230 nm due to the reduction of NAD catalized by hexokinase with the glucose substrate before and after chemotherapy 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, USA).

A simple exponential model of glucose consumption was then fitted to the data with the Systat program (Systat Inc. Evanston, IL, USA). 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 $PtCl_2$ (trans-d-dach) is a yellow crystal and water insoluble. Water soluble Pt-dinitrate (trans-d-dach) is prepared by replacement of Cl with nitrate. Final products of and [Pt(trans-d-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 (Table 1) is presented. The results of IR spectrum and the functional band of this compound are shown in Table 2. ^{13}C-NMR chemical shift and coupling constants are exhibited in Table 3.

Antitumor activity

Antitumor activity determination for cisplatin and PC aganist five carcinoma cell lines is shown using MTT assay.

Table 1. Result of elemental analysis of platinum(II) complex

Compound	Calculated(%)			Found(%)		
	H	C	N	H	C	N
PC	4.75	45.24	6.59	4.68	45.10	6.62

PC: [Pt(trans-d-dach)(DPPE)]-$2NO_3$

Table 2. IR spectra of DPPE and it's mixed ligand platinum(II) complex

Compounds	νHN	νCH(Phenyl)	δNH	νP-C(Phenyl)	$\nu NO_3(cm^{-1})$
DPPE		3067(W)		1432(VS)	
PC	3450 3192	3053(W)	1592	1441(VS)	1382 819

PC: [Pt(trans-d-dach)(DPPE)]-$2NO_3$

Table 3. ^{13}C-NMR spectra of DPPE and it's mixed ligand platinum(II)complex

Compounds	Phenyl group				Bidging CH_2	Diamine moiety			solvent
	δC_1(J P-C)	$\delta C_{2,6}$(J P-C)	$C_{3,5}$(J P-C)	δC_4	δC_7	$\delta C'_{1,2}$	$\delta C'_{3,6}$	$\delta C'_{4,5}$	
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(trans-d-cach)	a)	134.6(t,7.0)	192.4(t,6.4)	133.1(s)	24.3(s)	60.8(s)	31.6(s)	24.1(s)	DMSO
(DPPE)]-$2NO_3$		132.2(t,8.6)	126.3(t,4.0)	132.4(s)					

a): Resonance not osbserved

dach: 1,2-diaminocyclohexane δ: ppm from TMS

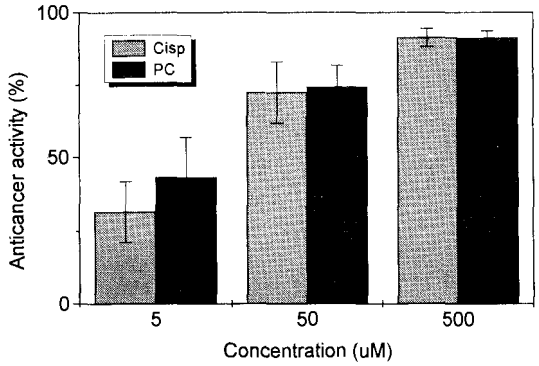

Fig. 1. Anticaner activities of Pt(II) complexes on the P-388 leukemia cells.
Cisp: Cisplatin
PC: pPt(trans-d-dach)(DPPE)]-2NO_3
dach: 1,2-diaminocyclohexane
DPPE: 1,2-Bis(diphenylphosphino)ethane

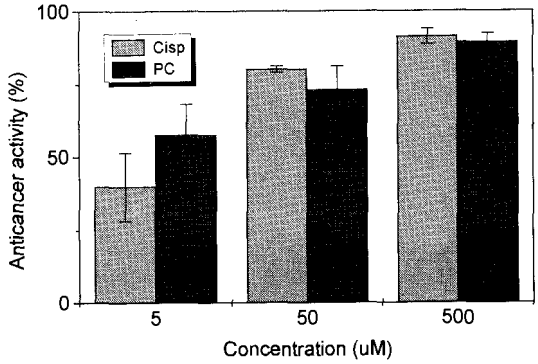

Fig. 2. Anticancer activities of Pt(II) complexes on the L-1210 leukemia cells.
Cisp: Cisplatin
PC: pPt(trans-d-dach)(DPPE)]-2NO_3
dach: 1,2-diaminocyclohexane
DPPE: 1,2-Bis(diphenylphosphino)ethane

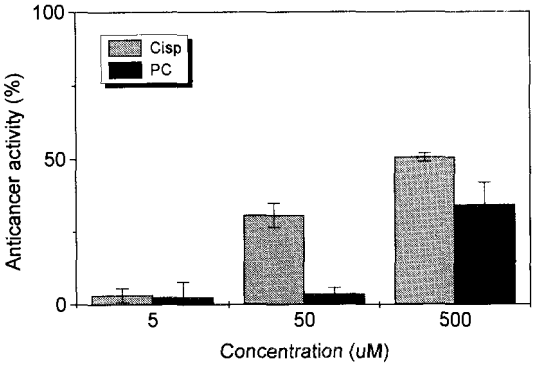

Fig. 3. Anticancer activities of Pt(II) complexes on the M-14 melanoma cells.
Cisp: Cisplatin
PC: pPt(trans-d-dach)(DPPE)]-2NO_3
dach: 1,2-diaminocyclohexane
DPPE: 1,2-Bis(diphenylphosphino)ethane

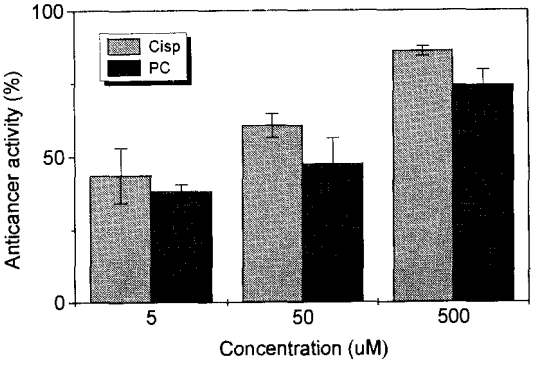

Fig. 4. Anticancer activities of Pt(II) complexes on the SKOV-3 human ovarian cancer cells.
Cisp: Cisplatin
PC: pPt(trans-d-dach)(DPPE)]-2NO_3
dach: 1,2-diaminocyclohexane
DPPE: 1,2-Bis(diphenylphosphino)ethane

Fig. 1 shows the result obtained after exposure of 5, 50 and 500 μM aganist P-388 leukemia cell-line. PC showed concentration-dependent increase in antitumor activity, PC exhibited significant antitumor activity (cytotoxicity index, CI: 43. 2% for 5 μM) and as active as that of cisplatin at 500 μM. The antitumor activity of PC showed only 74.3% and 91.3% of CI at 50 μM and 500 μM, respectively aganist P-388 leukemia cells.

Fig. 2 shows the results obtained when this drug was exposured to L-1210 mouse lymphocytic leukemia cell line. Antitumor activity of these Pt(II)-complex aganist L-1210 is also dependent on concentration and quite comparable to that of cisplatin. Cisplain and PC did not show any signifcant antitumor activity aganist M14 melano-

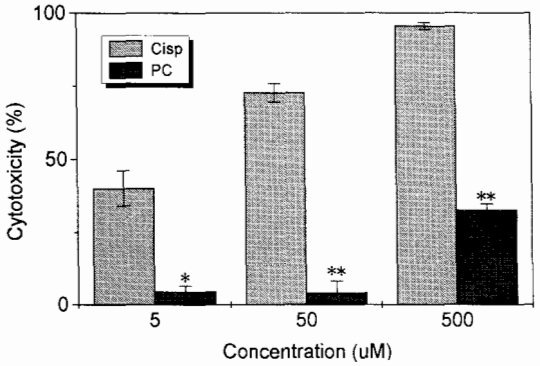

Fig. 5. Cytotoxic activities of Pt(II) complexes on the proximal tubule cells of rabbit kidney.
Cisp: Cisplatin
PC: pPt(trans-d-dach)(DPPE)]-$2NO_3$
dach: 1,2-diaminocyclohexane
DPPE: 1,2-Bis(diphenylphosphino)ethane

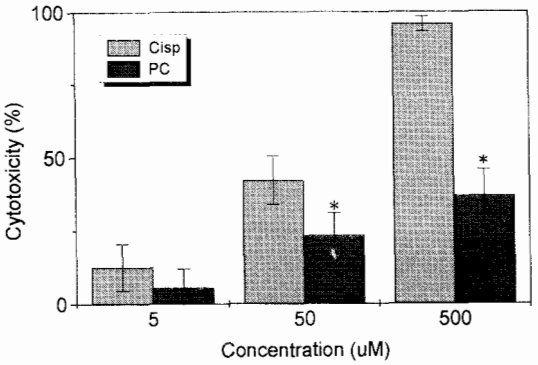

Fig. 6. Cytotoxic activities of Pt(II) complexes on the renal cortical cells of human kidney.
Cisp: Cisplatin
PC: pPt(trans-d-dach)(DPPE)]-$2NO_3$
dach: 1,2-diaminocyclohexane
DPPE: 1,2-Bis(diphenylphosphino)ethane

Table 4. Effect of platinum complex on ^{3}H-thymidine incorporation into primary cultured proximal tubule cells of rabbit kidney

Group	^{3}H-Tymidine (cpm/10^5cells)	Uptake Rate (%)
Control	598.3±75.15	100.0
Cisplatin	9.0±3.46	1.5
PC	409.7±68.38	68.5

Concentration of Pt-complex in cultured medium: 5×10^{-5}M
PC: [Pt(trans-d-dach)(DPPE)]$-2NO_3$
Values are means±S.E.
All the incoorporations were determined in triplicate.

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

Group	^{3}H-Tymidine (cpm/10^5cells)	Uptake Rate (%)
Control	621.3±56.01	100.0
Cisplatin	8.7±5.14	1.5
PC	275.3±51.24	44.3

Concentration of Pt-complex in cultured medium: 5×10^{-5}M
PC: [Pt(trans-d-dach)(DPPE)]$-2NO_3$
Values are means±S.E.
All the incoorporations were determined in triplicate.

ma cell line up to 500 μM (Fig. 3). These results indicate that M14 cell line is resistant to all these agents.

Fig. 4 shows the results when PC was exposured to SK-OV3 human ovarian adenocarcinoma cell lines. Antitumor activities of PC anainst SK-OV3 exhibited 74.3% (CI) at 500 μM concentration.

Nephrotoxicity

1) Rabbit kidney proximal tubules cells

The cytotoxicities of cisplatin and PC aganist rabbit kidney proximal tubular cells as determination by MTT assay are shown in Fig 5.

PC (CI: 4.5% showed less cytotoxic at 5 μM as compared with that of cisplatin (CI: 39.2%. At a concentration of 50 μM and 500 μM, PC showed 3-7 fold less cytoltoxic 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 4. PC showed 68.5%, respectively of [^{3}H]-thymidine uptake as compared with that of cisplatin (1.5%) at

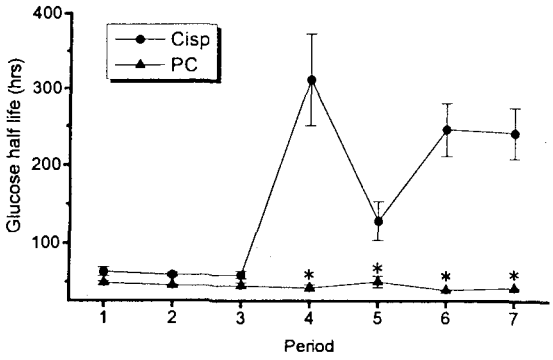

Fig. 7. Nephrotoxicity on 3 weeks histocultured human kidney. Toxicity was measured by glucose consumption. Each drug exposed for 72 hrs with 50 uM in concentration.
Cisp: Cisplatin
PC: pPt(trans-d-dach)(DPPE)]-2NO_3
dach: 1,2-diaminocyclohexane
DPPE: 1,2-Bis(diphenylphosphino)ethane

500 μM. This result indicates that cytotoxicity of PC was significantly less than that of cisplatin and [^{3}H]-thymidine uptake assay is more sensitive than MTT test.

2) Human kidney cortical cells

PC showed less cytotoxicity (CI: 23.4%) as compared with that of cisplatin (CI: 42.2%) at 50 μM (Fig. 6).

Table 5 shows the results obtained by [^{3}H]-thymidine uptake in primary cultured human kidney renal cortical cells. [^{3}H]-thymidine uptake in primary cultured human kidney renal cortical cells. [^{3}H]-thymidine incorporation is significantly inhibited by cisplatin (1.5%) as compared with that of PC (44.3%).

3) Human kidney cortical tissues

In glucose consumption, one period is defined as more than 3 times mesurement per day in 4 weeks histoculture of human kidney cortex tissue.

The half-life of glucose before adding of new synthetic Pt(II)-complex is approximately 23.3-48. 8 hr and does not show any statistical significance.

However, half-life of glucose was significantly increased at 4 period, and then showed more than 240 hr at 5,6 periods. However, the effect of PC was less marked than cisplatin (Fig. 7).

DISCUSSION

The platinum coordination complexes are cytotoxic agents that were first identified by Rosenberg et al. (1965). The inhibitory effects on of inorganic platinum-containing compounds in the presence of ammonium and chloride ions. cis-Diaminedichloroplatinum (II) (cisplatin) was found to be the most active of Pt (II) 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 maliganant tumors. However, its clinical use is often complicated 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 platinum containing compounds which have more favorable therapeutic indices and circumvent resistance.

The structure-activity relationships 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 1,2-diaminocyclohexane (dach) (Cleare and Hoeschele, 1973; Connors *et al.*, 1972; Gale *et al.*, 1974; Ridgway *et al.*, '977). Several dach compounds are existed 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 sifnificant toxic action on renal function (Odenheimer and Wolf, 1982; Appleton *et al.*, 1989; Alden and Repta, 1984).

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 (1980) noted that the distal tubule is the most consistently damaged region in human kidney. Furthermore, a number of investigators (Proter *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 routine 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 nephrotoxocity by diluting its tubular urinary concentration rather than by altering its half-life, plasma clearance or total urinary excretion.

New Pt (II)-containing analogues have generally been screened for antitumor activity and nephrotoxicity using several cancer cell lines (L1210 leukemia, P-388 leukemia, M-14 melanoma, and SK-OV3 ovarian cancer cells) and human/rabbit kidney normal cells, repectively. New synthetic Pt (II)-complex, PC exhibited significant antitumor activity aganist P-388, L-1210 and SK-OV3. However, the M-14 cells were somewhat resistant to ciaplatin and PC.

A criteria for antitumor activity in vitro is generally expressed in cytotoxicity index in P-388 and L-1210 and more than 50% in cytotoxicity index is accepted as positive antitumor drugs. PC showed comparable antitumor activity to cisplatin. 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 diaminocyclohexane 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 tubule epithelial cell-line) is a good model to evaluate nephrotoxicity induced by cisplatin in vitro. These studies using primary cultured 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 paramater 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 dimensinal culture method (Freman and Hoffman, 1986; Chang *et al.*, 1982).

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

REFERENCES

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

Appleton TG, Conner JW, Hall JR and Prenzler PD: *NMR study of the reactions of the cis-diammine-dichloroplatinum (II) cation with glutathione and amino acids containing a thiol group. Inorg Chem 28: 2030, 1989*

Bodenner DL, Dedon PC, Keng PC, Katz JC and Borch

RF: *Selective protection against cis-diamminedichloroplatinum (II) induced toxicity in kidney, gut, and bone marrow by diethyl-dithiocarbamate. Cancer Res 46(6): 2751-2755, 1986*

Bosanquet AG: *An assessment of a short term tumor chemosensitivity assay in chronic lymphocytic leukemia. Br J Cancer 47: 781-789, 1983*

Burchenal JH, Irani G, Kern K, Lokys L and Turkevich J: *1,2-Diaminocyclohexane platinum derivatives of potential clinical value. Recent Results Cancer Res 74: 146-155, 1980*

Burchenal JH, Kalaher Dew K and Lokys L: *Rationale for the develpment of platinum analogs. Cancer Treat Res 63: 1493-1498, 1979*

Burchenal JH, Kalaher K, O'Toole T, and Chisholm J: *Lack of cross resistance between certain platinum coordination compounds in mouse leukaemia. Cancer Res 37: 3455-3457, 1977*

Canetta R, Rozencweig M, Wittes RE and Schacter LP: *Platinum coordination complexes in cancer chemotherapy: an historical perspective. Cancer chemotherapy: challenges for the future. Excerpta Med Int Congr Ser 5: 318-323, 1990*

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

Chung SD, Alvi N, Livingston D, Hiller S and 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*

Cleare MJ and Hoeschele JD: *Antitumor platinum compounds: Relationship between structure and activity. Platinum Metals Review 17: 2, 1973*

Colombo N, Sartori E, Landini F, Favalli G, Vassena L, Zotti L, Mstermann E, Franks CR, Pecolelli S and Mangioni C: *Phase II study of platinum analog tno-6 in patient with advanced ovarian cancer. Cancer Treat Rep 70: 793-794, 1986*

Connors TA: *Antitumor effects of platinum complexes in experimental animals In: Connors TA, Roberts JJ eds: Platinum coordination complexes in cancer chemotherapy. New York Springer-Verlag 113, 1974*

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

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

Cvitkovic E, Spaulding J and Bethune V: *Improvement of cis-diamminechloroplatinum (NCS-119875): therapeutic index in an animal model. Cancer 39: 1357, 1977*

Dentino M, Luft FC, Yum MN, Williams SD and Einhorn LH: *Long term effect of cis-diamminedichloride platinum (CDDP) on renal function and structure in man. Cancer 41: 1274-1281, 1978*

Dobyan DC, Levi J, Jacobs C, Kosek J and Weiner MW: *Mechanism of cis-platinum nephrotoxocity: II. Morphologic observations. J Pharmacol Exp Ther 213 (#): 551-556, 1980*

Einhorn LH and Donohue J: *cis-Diamminedichloroplatinum, vinblastine and bleomycin combination chemotherapy in disseminated cancer. Ann Intern Med 87: 293, 1977*

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

Glover D, Glick JH, Weiler C, Fox K, Turrisi A and Kligerman MM: *Phase I/II trials of WR-2721 and cis-platinum. Int J Radiat Oncol Biol Phys 12(8): 1509-1512, 1986*

Glover D, Glick JH, Weiler C, Yuhas J and Kligerman MM: *Phase I trials of WR-2721 and cis-platinum. Int J Radiat Oncol Biol Phys 10(9): 1781-1784, 1984*

Gonzales-Vitale JC, Hayes DM, Cvitkovic E and Sternberg SS: *The renal pathology in clinical trials of cis-platinum (II) diamminedichloride. Cancer 39: 1362-1371, 1977*

Gottieb JA and Drewinko B: *Review of the current clinical status of platinum coordination complexes in cancer chemotherapy. Cancer Chemother Rep 59: 621, 1975*

Graeff A de, Slebos RJC and Rodenhuis S: *Resistance to cisplatin and analogues: mechanisms and potential clinical implications. Cancer Chemother Pharmacol 22: 325-332, 1988*

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

Harrap KR, Jones M, Wilkinson CR, Clink CJ, Sparrow S, Mitchley BCV, Clake S and Veasey A: *Antitumor toxic and biochemical properties of cisplatin and eight other platinum complexes. In: Prestayko AW, Crooke ST, Carter SK (eds) Cisplatin-current status and new developments. Academic Press New York pp. 193-212, 1980*

Hiby DJ, Wallace HJ and Holland JF: *cis-Diamminedichloroplatinum (NCS-119875) a phase I study. Cancer Chemother Rep 57: 459, 1973*

Hill JM, Loeb E, MacLellan A, et al: *Clinical studies of platinum coordination compounds in the treatment of various malignant disease. Cancer Chemother Rep 59: 647, 1975*

Holland JF, Bruckner HW, Cohen CJ, Wallach RC, Gusberg SB, Greenspan EM and Goldberg J: *Cisplatin therapy of ovarian cancer. In: Prestayko AW,*

Crooke ST, Carter SK (eds) Cisplatin-durrent status and new develpments. Academic Press New York pp. 383-392, 1980

Inagaki K and Kidani Y: *Differences in binding of (1,2-cyclohexanediamine) platinum (II) isomes with d(GPG). Inorg Chem 25: 1, 1986*

Jacobs C, Kalman SM, Tretton M and Weiner MW: *Renal handling of cis-diamminedichloroplatinum (II). Cancer Treat Rep 64: 1223, 1980*

Jones BR, Bhalla RB, Mladek J, Kaleya RN, Gralla RJ, Alcock NW, Schwartz MK, Young CW and Reindenberg MM: *Comparison of methods of evaluating nephrotoxicity of cis-platinum. Clin Pharmacol Ther 27(4): 557-562, 1980*

Jones MM, Basinger MA, Craft WD, Domingo JL and Liobet JM: *Control of some aspects of cis-platinum nephrotoxicity. Arch Toxicol 59(3): 167-171, 1986*

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

Kelsen DP, Scher H, Alcock N, Leyland-Jone sB, Donner A, Williams L, Greene G, Burchenal JH, Tan C, Philips JS and Young CW: *Phase clinical trial and pharmacokinetics of 4'-carboxyphthalato (1,2-diaminocyclohexane) platinum (II). Cancer Res 42: 4831-4835, 1982*

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

Kociba RJ, Slieight SD and Rosenbegr B: *Inhibition Dunning ascitic leukemia and Walker 256 carcinosarcoma with cis-diamminedichloroplatinum (NCS-119875). Cancer Chemother Rep 57: 325, 1970*

Kociba RJ and Sleight SD: *Acute toxicologic and pathologic effects of cis-diamminedichloroplatinum (NCS-119875) in the male rat. Cancer Chemother Rep 55: 1, 1971*

Krakoff IH: *Nephrotoxicity of cis-diamminedichloroplatinum (II). Cancer Treat Rep 63: 1523, 1979*

Levi J, Jacobs C, Kalman SM, McTigue M and Weiner MW: *Mechanism of cis-platinum nephrotoxicity: I. Effect of sulfhydryl groups in rat kidney. J Pharmacol Exp Ther 213(3): 545-550, 1980*

Lippman AJ, Helson C, Helson L and Kradoff IH: *Clinical trials of cis-diaminodichloroplatinum (NSC-119875). Cancer Chemother Rep 57: 191, 1973*

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

Meijer S, Mulder NH, Sleijfer DT, de Jong PE, Sluter WJ, Schraffordt KH and van der Hem GK: *Nephrotoxicity of cis-diammine-dichloride platinum (CDDP) during remission-induction and maintenance chemotherapy of testicular carcinoma. Cancer Chemother Pharmacol 8(1): 27-30, 1982*

Mortine TJ and Borch RF: *Quiescent LLC-PK1 cells as a model for cis-diamminedichloroplatinum (II) nephrotoxicity and modulation by thio resucue agents. Cancer Res 48: 6017-6024, 1988*

Noji M, Okamoto K and Kidani Y: *Reaction of conformation to antitumor activity of platium (II) complexes of 1,2-cyclohexanediamine and 1-(aminomethyl) cyclohexylamine isomers against leukemia P388. J Med Chem 24: 508, 1981*

Odenheimer B and Wolf W: *Reactions of cisplatin with sulfur containing amino acids and peptides (I): cytosine and glutathione. Inorg Chim Acta L41: 66, 1982*

Porter GA and Bennett WM: *Toxic nephropathies: In the kidney (2nd. ed.). W.B. Saunders Co. Philadelphia pp. 2045-2108. 1981*

Ridgway HJ, Speer RJ and Hall JM: *Analogs of sulfato 1,2-diaminocyclohexane platinum (II): Modifications in leaving ligands. J Clin Hematol Oncol 7: 220, 1977*

Rosenberg B, Van Camp L, Grimley EB and Thomson AJ: *The inhibiton of growth or cell division in Escherichia coli by different ionic species of platinum (IV) complexes. J Biol Chem 242: 1347, 1967*

Rosenberg B, Van Camp L and Krigas TL: *Inhibition of cell division of Escherichia coli by electrolysis products from a platinum electrode. Nature 205: 698, 1965*

Rosenberg B, Van Camp, L, Krosko JE and Mansour VH: *Platinum compounds: a new class of potent cntitumor agnets. Nature 223: 385, 1969*

Schaeppi U, Hetman IA and Fleischman RW: *cis-Dichlorodiammineplatinum (NSC-119875): preclinical toxicologyic evaluation of intravenous injection in dogs, monkeys and mice. Toxicol Appl Pharmacol 25: 230, 1973*

Scher HI, Kelsen D, Kalman L, Jones L, Burchenal J and Gralla R: *Phase II trial of 1,2-diaminocyclohexane (4-carboxyphthalato) platinum (II) (DACCP) in non-small cell lung cancer. Cancer Chemother Pharmocol 12: 101-103, 1984*

Sherman SE and Lippard SJ: *Structual aspects platinum anticancer drug interactions with DNA. Chem Res 87: 1153, 1987*

Sorensen PG, Nissen MH, Groth S, and Roth M: *Beta-2-microglobulin excretion: an indicator of long term nephrotoxicity during cis-platinum treatment. Cancer Chemother Pharmacol 14(3): 247-249, 1985*

Tashiro T: *Antitumor activity and mechanism of anticancer chemotherapeutic platinum complexes. J Jap Chemistry 4: 648, 1988*

Tirelli AS, Colombo N, Cavanna G, Mangioni C and Assael BM: *Follow-up study of enzymuria and beta-*

2-microglobulinuria during cis-platinum treatment. Eur J Clin Pharmacol 29(3): 313-138, 1985

Walker EM and Gale GR: *Methods of recution of cisplatin nephrotoxicity. Ann Clin Lab Sci 11: 397, 1981*

Ward JM and Fauvie KA: *The nephrotoxic effects of cis-diaminedichloroplatinum (II) (NSC-119875) in male F344 rats. Toxicol Appl Pharmacol 38: 535, 1976*

Ward JM, Young DM, Fauvie KA, Wolpert MK, Davis R and Guarino AM: *Comparative nephrotoxicity of platinum cancer chemotherapeutic agent. Cancer Chemother Rep 60(1675, 1976)*

Wilkoff LJ, Dulmadge EA, Trader MW, Harrison SD and Griswold DP: *Evaluation of trans-tetrachloro-1, 2-diaminocyclohexane platinum (IV) in murine leukaemia L1210 resistant and sensitive to cis-diaminedichloroplatinum (II). Cancer Chemother Pharmacol 20: 96-100, 1987*

Wolf W and Manaka RC: *Synthesis and distribution of 195mPt cis-dichlorodiamine platinum (II). J Clin Hematol Oncol 7: 79-95, 1976*

=국문초록=

새로운 Platinum (II) Complex [Pt(II)(trans-d-dach(DPPE)] $(NO_3)_2$ 의 항암효과 및 신독성

경희대학교 의과대학 약리학교실 및 비뇨기과학교실*
경희대학교 약학대학 약화학교실**

정지창 · 이문호 · 장성구* · 노영수**

일부 malignant tumor에 Pt-complex의 임상 응용 과정에서 신장독성등의 심한 부작용이 문제점으로 지적되고 있다.

이 연구에서는 기존의 cisplatin보다 항암효과는 우수하면서, 부작용을 감소시킨 새로운 Pt-complex의 개발에 역점을 두었다. 본 연구에서 합성한 Pt (II) complex는 carrier ligand로서 1, 2-diaminocyclohexane(dach)을 사용하였고, leaving group으로는 diophosphine류인 1,2-bisdiphenylphosphinoethane (DPPE)을 도입하였으며, 물에 대한 용해도를 높이기 위해 dinitrate로 만들었다.

새로이 합성한 [Pt(II)(trans-d-dach)(DPPE)]$(NO_3)_2$는 원소 분석, IR 및 ^{13}C-NMR 분석 data에 의하여 위의 물질임이 확인되었다.

MTT assay method에 의한 항암활성 연구를 통하여 P-388, L-1210 lymphocytic leukemia cell과 SK-OV3 난소암세포에서 항암효과가 인정되었으며, 이 항암효과는 대조 약물로 사용된 cisplatin과 유사하였다.

토끼의 신세뇨관 세포와 인체의 신피질 세포를 이용한 cytotoxity 및 thymidine 섭취율과 인체 신피질 조직 배양을 이용한 glucose consumption 실험을 통하여 모두 cisplatin보다 신장독성이 현저히 감소되었다.

이상의 결과로 보아 Pt(II) complexes는 carrier ligand와 leaving group의 선택에 따라 항암활성의 증가와 신독성의 감소를 일으키는 요인으로 보여지며, 이 연구에서 만들어진 Pt(II) complex는 앞으로 다각적인 검토를 거쳐 새로운 항암화학요법제로 개발될 가능성이 있을 것으로 생각된다.