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A New Class of Platinum (II) Complexes [Pt (trans-l-dach) (DPPP)] $2NO_3$ and [Pt (trans-l-dach)(DPPE)] $2NO_3$ Exhibiting Antitumor Activity and Nephrotoxicity

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ABSTRACT

Pt-complexes is currently one of the most compounds used in the treatment of solid tumors. However, its used 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 analogues containing 1, 2-diaminocyclohexane (dach) as carrier ligand and 1, 3-bis (diphenylphosphino) propane (DPPP)/1, 2-bis (diphenylphosphino) ethane (DPPE) as a leaving group. Furthermore, nitrate was added to improve the solubility. A new series of (KHPC-001) [Pt (trans-l-dach)(DPPP)] $2NO_3$ and (KHPC-002) [Pt (trans-l-dach)(DPPE)] $2NO_3$ were synthesized and characterized by their elemental analysis and by various spectroscopic techniques [infrared (IR), ^{13}carbon nuclear magnetic resonance (NMR)].

KHPC-001 and KHPC-002 demonstrated acceptable antitumor activity aganist P-388, L-1210 lymphocytic leukemia cells and significant activity as compared with that of cisplatin. The toxicity of KHPC-001 and KHPC-002 was found quite less than that of cisplatin using MTT, [^{3}H] thymidine uptake and glucose consumption tests in rabbit proximal tubule cells and human kidney cortical cells.

Key Words: Platinum complexes, Antitumor activity, Nephrotoxicity, Rabbit proximal tubule cells, Human kidney cortical cells

INTRODUCTION

Cis-Diaminedichloroplatinum (cisplatin) is one of the first-line chemotherapeutic agents for the treatment of ovarian carcinoma, testicular cancer cancer of the head and neck, and bladder cancer (Holland *et al.*, 1980). The antineoplastic activity of cisplatin is attributed to its preferential reaction with N-7 atoms on the guanine bases in DNA; such reaction ultimately form compounds in which both chlorides are replaced by nucleic acid groups (Sherman and Lipard, 1987). However, its usefulness is compromised by its propensity to cause several dose-limiting toxicities, including nephrotoxicity, nausea, vomiting, neurotoxicity, ototoxicity, and myelosuppression (Harrap *et al.*, 1980), and its potential to induce resistance in otherwise responsive tumor types (Graeff *et al.*, 1988). Nephrotoxicity is the observations that the kidney accumulates and retains platinum to a greater extent than other organs, and it is the kidney that is the predominant excretory organ for

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platinum complex (Litter *et al.*, 1976). The prominent contribution thus far, in over 20 years of study, has been the discovery of the 1, 2-diaminocyclohexane (DACH) carrier ligand (Burchenal *et al.*, 1979). These DACH complexes retain activity in cisplatin-acquired resistant murine leukemias, both in vitro (Burchenal *et al.*, 1980 and 1987) and in vivo (Wilkoff *et al.*, 1987). Some DACH complexes [e.g., 1, 2-diaminocyclohexane (4-carboxyphthalato) platinum (II)] and [1, 1-diaminomethylcyclohexane sulfato platinum (II)] have entered Phase I/II clinical trials but have sequently been dropped, primarily due to unacceptable toxicity (Canetta *et al.*, 1990; Colombo *et al.*, 1982; Kesen *et al.*, 1983; Scher *et al.*, 1984).

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 several new platinum analogues containing DACH as carrier ligand and 1, 3-bis (diphenylphosphino) propane (DPPP)/1, 2-bis (diphenylphosphino) ethane (DPPE) as a leaving group. The present study reports on the synthesis of new platinum complexes and their nephrotoxicity and antitumor activity evaluated with cancer cell line or primary rabbit/human kidney cells as a compared with those of cisplatin.

MATERIAL 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-1-1, 2-cyclohexanediamine was kindly supplied by Dr. Kidani; Department of Pharmacy, University of Nagoya (JAPAN). This trans-l-dach was separated from trans-dl-dach purchased from Aldrich Chemical Co. (Milwaukee, WI).

The chemical structure of platinum analogues were presented:

KHPC-001 [Pt (trans-l-dach)(DPPP)] $2NO_3$

KHPC-002 [Pt (trans-l-dach)(DPPE)] $2NO_3$

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

Synthesis of platinum (II) complexes

(1) (trans-l-1, 2-cyclohexanediamine) dichloro platinum (II) [Pt (trans-l-dach) Cl_2]: To a solution of K_2PtCl_4 (420 mg, 1.02 mM) in H_2O (30 ml) was treated dropwise a solution of trans-l-dach. 2HCl (190 mg, 1.01 mM). The mixture was adjusted to pH 6.5 by titration with 5% NaOH solution and stirred for 30 min at room temperature. The yellow crystals was were formed and filtered. The filtrate was dried in vacuum evaporation; yield 468 mg.

(2) (trans-d-1, 2-cyclohexanediamine) dichloro platinum (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). Next procedure was followed according (1); yield 470 mg.

(3) (trans-l-1, 2-cyclohexanediamine) dinitrate platinum (II)-[Pt (trans-l-dach)$(NO_3)_2$]: To a suspension of Pt (trans-l-dach) Cl_2 (380 mg, 1 mM) was treated stepwise a solution of $AgNO_3$ (340 mg, 1.0 mM) in water (10 ml). The reaction mixture was stirred for 24 hr at room temperature. The reaction product of AgCl was filtered off. The filtrate was concentrated under reduce pressure and dried with lysophilization; yield 296 mg.

(4) (trans-1, 2-cyclohexanediamine) dinitrate-platinum (II)-[Pt (trans-d-dach)$(NO_3)_2$]: The solution of Pt (trans-d-dach) Cl_2 380 mg (1 mM) was suspended in 10 ml of H_2O, and followed the same procedure of (3); yield 310 mg.

(5) {1, 3-Bis (diphenylphosphino) propane} (trans-l-1, 2-cyclohexanediamine) Pt (II) nitrate-[Pt (trans-l-dach)(DPPP)] $(NO_3)_2$ H_2O: To a solution of Pt (trans-l-dach)$(NO_3)_2$ (433 mg, 1 M) in H_2O (10 ml) was added a solution of DPPP (410 mg, 1 mM) in acetone (20 ml). The mixture was stand for 1 hr and evaporate under reduced pressure. The yellow crystals formed and dried with lysophilization. The product was recrystalized from H_2O; yield 417 mg.

Cell culture

Cell culture environment; Primary rabbit kidney proximal tubule cells were maintained in a 37℃, 5% CO_2 humidified environment in a serum-free basal medium supplemented with 3 growth supplements, 5 μg/ml insulin, 5 μg/ml transferrin and 5×10^{-8} M hydrocortisone (Jung *et al.*, 1992). The basal medium, a serum-free modified DME/F12 medium, pH 7.4, was a 50:50 mixture of Dulbecco's Modified Eagle's Medium and Ham's F12 lacking hypoxanthine, thymidine, pyruvate, L-glutamine, L-glutamic acid, L-serine, and linoleic acid. The basal medium was further supplemented with 15 mM HEPES buffer (pH 7.4) and 20 mM sodium bicarbonate. Immediately prior to the use of the medium, the 3 growth supplements were added. Water utilized in medium preparation was purified by means of a Millique deionization system.

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.* (1982). 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 turned grey-black in color. Renal cortical slices were homogenized with 4 storkes of a sterile Dounce homogenizer (type A pestle), 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, and transfered into tissue culture dishes. Medium was changed one day after plating and every four days thereafter.

Primary human kidney cortical cell culture; Normal kidney tissue was freshly excised from patient undergoing abdominal operation. Kidney cortical was 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 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_2 (5×10^{-8} M) and fetal bovine serum (1 %). This suspensed medium was seeded on petri dish in an incubator at 37℃ maintaining highly humidifed atmosphere 5% CO_2/95% air. After 2 weeks incubation, the cells were confluent and used for experiments.

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). One gel was put in each well of six-well dishes three mililiters of Eagle's minimal essential medium (MEM)(GIBCO, Grand Island NY) supplemented with 10% fetal bovine serum (GIBCO) and 50 mg/ml gentamicin 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_2/95% air incubator at 37℃. The cultures underwent sterile media changes every 72 hr. Histoculture was continued up to 3 weeks after explantation. Specimens were exposed to media containing newly formed platinum complex and cisplatin for 72 hr. After drug treatment, the specimens were washed with phosphate buffered saline and fresh media. (Freeman and Hoffman, 1986; Chang *et al.*, 1992).

Antitumor activity

Mouse leukemia cancer cell line L-1210 and P-388 were cultured in 20 ml of RPMI medium supplemented with 100 mg/ml strptomycin/penicillin and 10% fetal calf serum (FCS) in incubators maintaining highly humidified 5% CO_2/95% air. M-14 melanoma cells were cultured under the same condition above explained except DMEM medium. 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.

Individual wells of 96-well tissue culture microtiter plate were inoculated with 0.1 ml of the appropriate media containing 10^5 cells. Platinum-complexes were added at various concentration. 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 was added and absorption was read at 630 nm and automatically recorded with ELISA. The control compound is cisplatin.

Nephrotoxicity

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 was 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, 50 and 500 μ M) and cultures were incubated for 48 hrs in an incubator maintaining highly humidified atmosphere of 5% CO_2/95% air. 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 solublized 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 kideny 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. Thereafter, [^{3}H]-thymidine (1 μCi/ml; specific radioactivity) was then added to each well, and cells were again incubated for 24 hr. 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 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 5000 TD).

Glucose consumption test; 50 μl of culture medium was taken every 24 hr for determination of medium glucose content in triplicate using the HK 20 assay kit from Sigma (St. Lousi, MO). Measurement were made by monitoring the change in optical density at 340 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).

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 $t1/2 = 0.693/s$, 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-l-dach) is a yellow crystal and water insoluble. Water soluble Pt-dinitrate (trans-l-dach) is prepared by replacement of Cl with nitrate. Final products of [Pt (trans-l-dach) (DPPP)] $2NO_3$ (KHPC-001) and [Pt (trans-l-dach) (DPPE)] $2NO_3$ (KHPC-002) are synthesized by mixing 1 : 1 ratio of DPPE/DPPP to above prepared compound.

The platinum complexes were submitted for elemental analysis prior to biological evaluation. Analytical data (Table 1) is presented. There is no significant difference between KHPC-001 and KHPC-002 in IR spectrum and the functional band of these compounds are shown in Table 2. The results of ^{13}C-NMR about free leaving group (DPPP and DPPE) and synthetic Pt (II)-complexes are shown in Fig. 1. ^{13}C-NMR chemical shift and coupling constants are exhibited in Table 3.

Table 1. Results of elemental analysis of platinum (II) complexes

Compounds	Calculated(%)			Found(%)		
	H	C	N	H	C	N
KHPC-001	4.90	45.89	6.49	4.92	45.68	6.38
KHPC-002	4.88	44.30	6.46	4.90	45.00	6.50

KHPC-001: [Pt (trans-l-dach)(DPPP)] $(NO_3)_2$
KHPC-002: [Pt (trans-l-dach)(DPPE)] $(NO_3)_2$

Table 2. IR spectra of DPPP, DPPE and their mixed ligand platinum (II) complexes

Compounds	vNH	vCH(Phenyl)	δNH	vP-C(Phenyl)	$vNO_3(cm^{-1})$
DPPP		3010(W)		1437(VS)	
KHPC-001	3430 3182	3081(W)	1640	1442(VS)	1384 820
DPPE		3067(W)		1432(VS)	
KHPC-002	3450 3192	3053(W)	1592	1441(VS)	1382 819

KHPC-001: [Pt (trans-l-dach)(DPPP)] $(NO_3)_2$
KHPC-002: [Pt (trans-l-dach)(DPPE)] $(NO_3)_2$

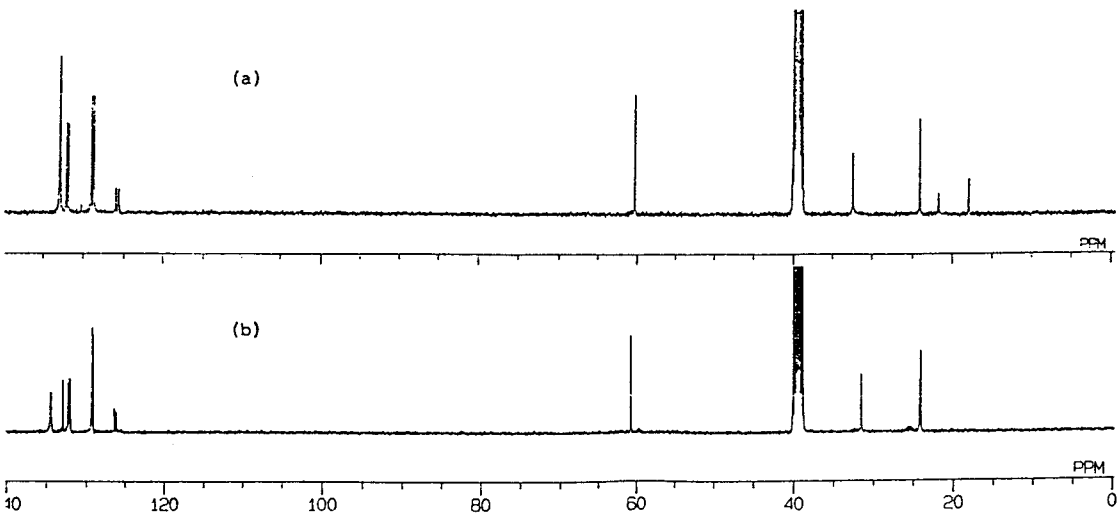

Fig. 1. ^{13}C-NMR spectra of [Pt (trans-l-dach)*L)] $(NO_3)_2$ in DMSO.
L=(a) DPPP; (b) DPPE

This evidence suggests the chemical structures shown in Fig. 2.

Antitumor activity

Antitumor activity determination for cisplatin, KHPC-001 and KHPC-002 aganist three carcinoma cell lines is shown using MTT assay. Fig. 3. shows the result obtained after exposure of 5, 50 and 500 μM aganist P-388 leukemia cell-line. Both KHPC-001 and KHPC-002 showed concentration-

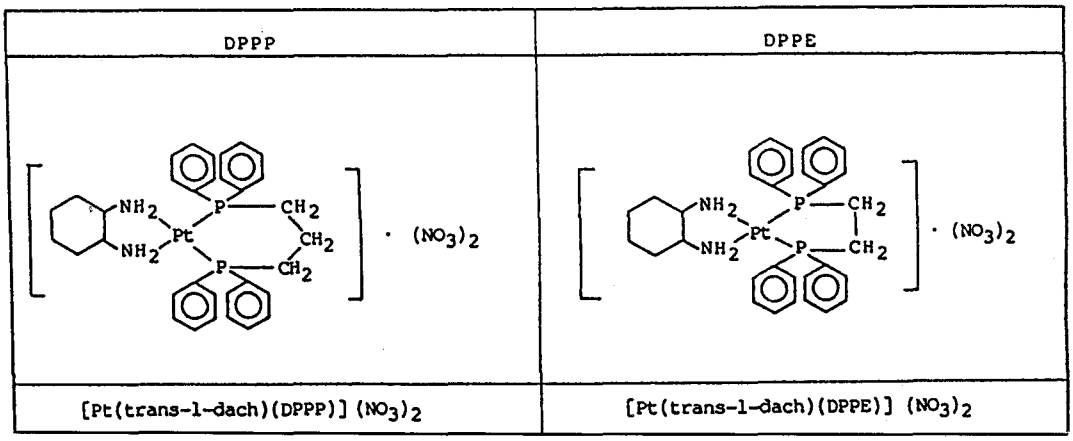

Fig. 2. Structures of Pt (II) complexes containing dach isomers and diphosphines (DPPP or DPPE).

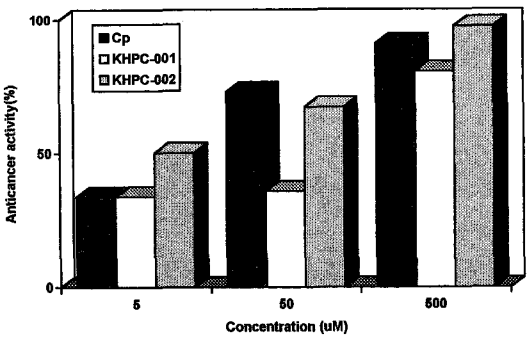

Fig. 3. Anticancer activities of platinum (II) complexes on the P-388 leukemia cells.
Cp: Cisplatin
KHPC-001: [Pt (trans-l-dach)(DPPP)]-2NO3
KHPC-002: [Pt (trans-l-dach)(DPPE)]-2NO3
*dach···1, 2-chclohexanediamine
*DPPP···1, 3-Bis (diphenylphosphino) propane
*DPPE···1, 2-Bis (diphenylphosphino) ethane

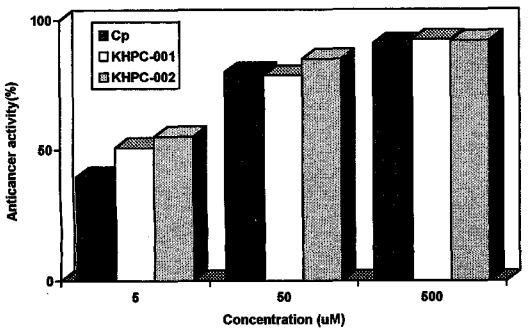

Fig. 4. Anticancer activities of platinum (II) complexes on the L-1210 leukemia cells.
Cp: Cisplatin
KHPC-001: [Pt (trans-l-dach)(DPPP)]-2NO3
KHPC-002: [Pt (trans-l-dach)(DPPE)]-2NO3
*dach···1, 2-cyclohexanediamine
*DPPP···1, 3-Bis (disphenylphosphino) propane
*DPPE···1, 2-Bis (diphenylphosphino) ethane

dependent increase in antitumor activity, especially KHPC-002 exhibited significant antitumor activity (cytotoxicity index, CI: 50.2% for 5 μM) and as active as that of cisplatin at 500 μM. The antitumor activity of KHPC-001 showed only 35.7 % and 33.8% of CI at 5 μM and 50 μM, respectively aganist P-388 leukemia cells, and then antitumor activity was only acceptable at 500 μM (CI: 80.6 %).

Fig. 4 shows the results obtained when these drugs were exposured to L-1210 mouse lymphocytic leukemia cell line. Antitumor activity of these Pt (II)-complexes aganist L-1210 is also dependent on concentration and quite comparable to that of cisplatin. Cisplatin, KHPC-001 and KHPC-002 did not show any significant antitumor activity aganist M14 melanoma cell line up to 500 μM (Fig. 5). These results indicate that M14 cell

line is resistant to all these agents.

Nephrotoxicity

Rabbit kidney proximal tubules cells: The cytotoxicities of cisplatin, KHPC-001 and KHPC-002 aganist rabbit kidney proximal tubular cells as determination by MTT assay are shown in Fig. 6. KHPC-001 and KHPC-002 (CI: 5.9% and 10.8%, respectively) showed less cytotoxic at 5 μM as compared with that of cisplatin (CI: 39.2%). At a concentration of 50 μM and 500 μM, KHPC-001 and KHPC-002 showed 2-fold less cytotoxic than that of cisplatin.

In addition to MTT assay, cytotoxicities were determined using [^{3}H]-thymidine uptake assay. Result using this assay was shown in Table 5. KHPC-001 and KHPC-002 showed 49.1% and 22.4 %, respectively of [^{3}H]-thymidine uptake as compared with that of cisplatin (1.5%) at 500 μM. This result indicates that cytotoxicities of KHPC-001 and KHPC-002 are significantly less than that of cisplatin and [^{3}H]-thymidine uptake assay is more sensitive than MTT test (up to 7-fold). We can not find any significant cytotoxicity between KHPC-001 and KHPC-002 in thymidine uptake.

Human kidney cortical cells: KHPC-001 and

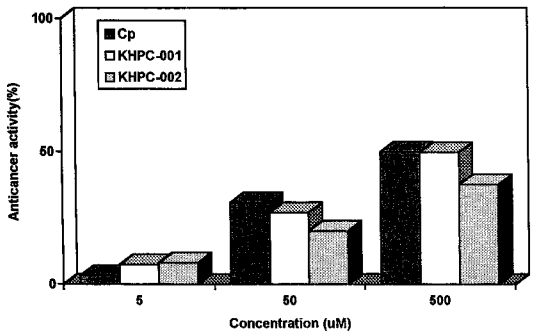

Fig. 5. Anticancer activities of platinum (II) complexes on the M-14 melanoma cells.
Cp: Cisplatin,
KHPC-001: [Pt (trans-l-dach)(DPPP)]-2NO3
KHPC-002: [Pt (trans-l-dach)(DPPE)]-2NO3
*dach···1, 2-cyclohexanediamine
*DPPP···1, 3-Bis (disphenylphosphino) propane
*DPPE···1, 2-Bis (diphenylphosphino) ethane

Table 4. Effect of platinum complexes on ^{3}H-thymidine incorporation into primary cultured proximal tubule 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
KHPC-001	293.7±32.03	49.1
KHPC-002	134.0±41.02	22.4

Concentration of Pt-complexes in culture medium: 5 $\times 10^{-5}$ M
KHPC-001: [Pt (trans-l-dach)(DPPP)] $2NO_3$
KHPC-002: [Pt (trans-l-dach)(DPPE)] $2NO_3$
Values are means±SE. All the incorporations were determined in triplicate.

Table 3. ^{13}C-NMR spectra of DPPP, DPPE and those mixed ligand Pt (II) complexes

Compounds	Phenyl group				Bridging CH_2		Diamine moiety			Solvent
	δC_1(Jp-c)	$\delta C_{2,6}$(Jp-c)	$\delta C_{3,5}$(Jp-c)	δC_4	δC_7	δC_8	$\delta C'_{1,2}$	$\delta C'_{3,6}$	$\delta C'_{4,5}$	
DPPP	138.5(t, 8.0)	133.5(t, 7.2)	128.2(t, 5.2)	131.4(s)	24.8(s)	17.8(s)				CD_2Cl_2
[PT (dach)[a]	b)	133.1(t, 6.9)	128.8(t, 9.2)	132.2(s)	24.1(s)	18.0(s)	60.4(s)	32.4(s)	21.8(s)	DMSO
(DPPP)] • $(NO_3)_2$	139.2(t, 7.5)	129.1(t, 8.8)	125.8(t, 9.2)	132.0(s)						
DPPE	b)	133.8(t, 8.8)	129.5(t, 3.5)	130.5(s)	25.1(s)					CD_2Cl_2
[Pt (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)	DMSO
(DPPE)] • $(NO_3)_2$		132.2(t, 8.6)	126.3(t, 4.0)	132.4(s)						

a): Trans-l-dach
b): Resonance not observed, δ: ppm from TMS

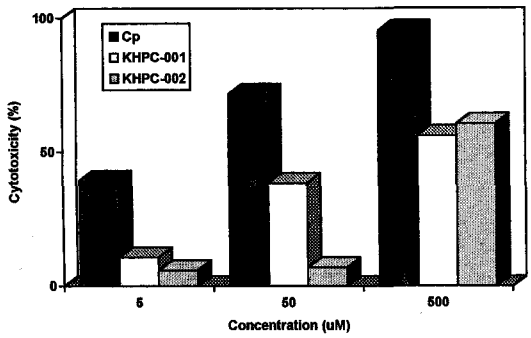

Fig. 6. Cytotoxic activites of platinum (II) complexes on the proximal tubule cells of rabbit kidney.
Cp: Cisplatin
KHPC-001: [Pt (trans-l-dach)(DPPP)]-2NO3
KHPC-002: [Pt (trans-l-dach)(DPPE)]-2NO3
*dach···1, 2-cyclohexanediamine
*DPPP···1, 3-Bis (disphenylphosphino) propane
*DPPE···1, 2-Bis (diphenylphosphino) ethane

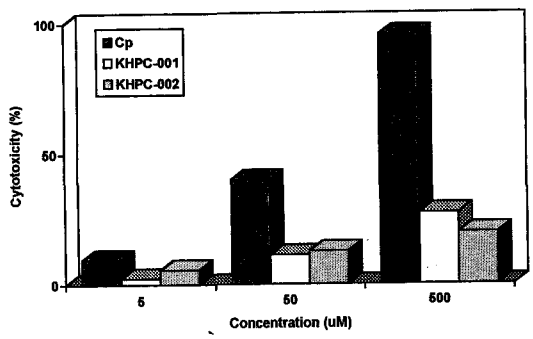

Fig. 7. Cytotoxic activities of platinum (II) complexes on the renal cortical cells of human kidney.
Cp: Cisplatin
KHPC-001: [Pt (trans-l-dach)(DPPP)]-2NO3
KHPC-002: [Pt (trans-l-dach)(DPPE)]-2NO3
*dach···1, 2-cyclohexanediamine
*DPPP···1, 3-Bis (disphenylphosphino) propane
*DPPE···1, 2-Bis (diphenylphosphino) ethane

Table 5. Effect of platinum 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
KHPC-001	282.7±25.01	45.5
KHPC-002	160.0±33.26	25.8

Concentration of Pt-complexes in culture medium: 5×10^{-5} M
KHPC-001: [Pt (trans-l-dach)(DPPP)] $2NO_3$
KHPC-002: [Pt (trans-l-dach)(DPPE)] $2NO_3$
Values are means±SE. All the incorporations were determined in triplicate.

KHPC-002 showed less cytotoxicity (CI: 27.5% and 19.9%, respectively) as compared with that of cisplatin (CI: 94.5%) at 50 μM (Fig. 7). Table 5 shows the results obtained by [^{3}H]-thymidine uptake in primary cultured human kidney renal cortical cells. [^{3}H]-thymidine incorporation is significantly inhibited by cisplatin (1.4%) as compared with that of KHPC-001 (45.5%) and KHPC-002 (25.8%).

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)-complexes is approximately 28-39.6 hr and does not show any statistical significance. However, half-life of glucose was significantly increased at 4 period, and then showed more than 190 hr at 5, 6 periods. However, the effect of KHPC-001 and KHPC-002 was less marked than cisplatin (Fig. 8).

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 overcomed by the development of the second-generation agent, carboplatin, there remains an unquestionable

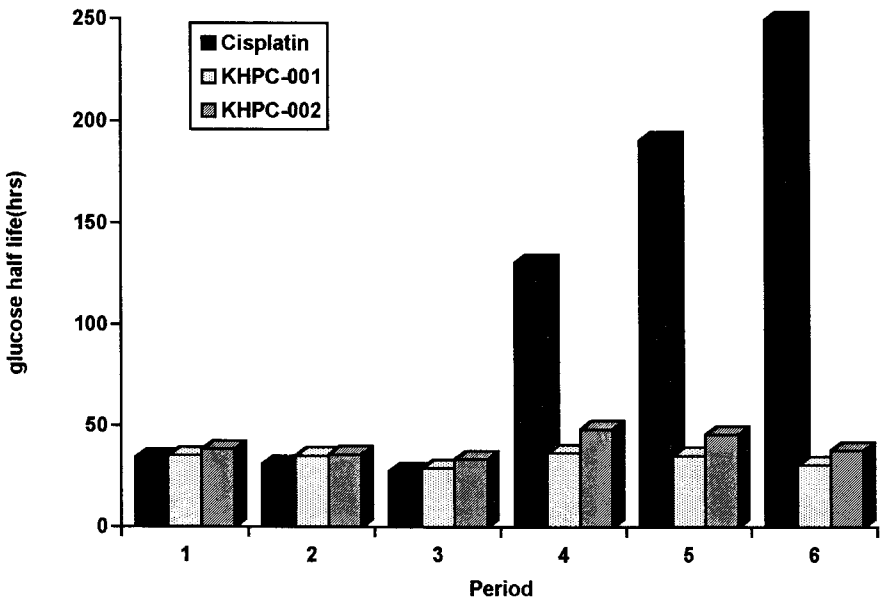

Fig. 8. Nephrotoxicity on 3 weeks histocultured human kidney.
Toxicity was measured by glucose consumption. Each drug exposed for 72 hrs with 0.5 mM in concentration.
KHPC-001: [Pt (trans-l-dach)(DPPP)] $2NO_3$
KHPC-002: [Pt (trans-l-dach)(DPPE)] $2NO_3$

need for further platinum containing compounds which have more favorable therapeutic indices and circumvent resistance. The structure-activity relationships were 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; Connore *et al*, 1972; Gale *et al*, 1974; Ridgway *et al*, 1977). Several dach compounds are existed such as cis-dach, trans-l-dach and trans-d-dach. Among these dach derivaties, 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 penetrate 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; 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 blood-flow in kidney or depletion of intracellular glutathione (Meijet *et al.*, 1982; Levi *et al.*, 1980). Dobyan *et al* (1980) have reported site-specific injury to the pars recta (S_3) segment of the proximal tubules. Gonzalez-Vitale *et al* (1980) noted that the distal tubule is the most consistenly 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 analogues have generally been screened for antiumor activity and nephroroxicity using several cancer cell lines (L1210 leukemia, P-388 leukemia and M-14 melanoma cells) and human/rabbit kidney cells, respectively. New synthetic Pt (II)-complexes, KHPC-001 ans KHPC-002 exhibited significant antitumor activity aganist P-388 and L-1210. However, the M-14 cells was somewhat resistant to cisplatin, KHPC-001 and KHPC-002.

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 postive antitumor drugs. KHPC-001 and KHPC-002 showed comparable antitumor activity to cisplatin. However, KHPC-002 demonstrated significant antitumor activity as compared with that of cisplatin at low concentration.

The comparison of antitumor activity between KHPC-001 and KHPC-002 exhibited similar in L-1210, whereas that of KHPC-002 showed almost 2-fold higher than that of KHPC-001 in P-388. Since the difference of chemical structure between KHPC-001 and KHPC-002 is only leaving group, it can be explained that dissociation time or rate is different after absorbing the compound in the cells. The results obtained here presented that KHPC-001 and KHPC-002 have less cytotoxic than cisplatin. This is conceivable that modification of the carrier ligand as a diaminocyclohexane and leaving group as a DPPP/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 apperance 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 are 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 kidney proximal tubule cells and human renal cortical cell culture system may have value in detecting potential nephrotoxicity and in studying their mechanism.

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=국문초록=

새로운 Platinum (II) Complex ([Pt (II)(trans-l-dach)(DPPP)] $(NO_3)_2$와 [Pt (II)(trans-l-dach)(DPPE)] $(NO_3)_2$의 항암효과 및 신독성에 관한연구

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일부 malignant tumor에 Pt-complex의 임상 응용 과정에서 신장독성등의 심한 부작용이 문제점으로 지적되고 있다. 이 연구에서는 기존의 cisplatin보다 함암효과는 우수하면서, 부작용을 감소시킨 새로운 Pt-complex의 개발에 역점을 두었다. 본 연구에서는 합성한 Pt (II) complex는 carrier ligand로서 1, 2-diaminocyclohexane (dach)을 사용하였고, leaving group으로는 diphosphine류인 1, 3-bis (diphenylphosphine)의 propane (DPPP) 및 ethane (DPPE)을 도입하였으며, 물에 대한 용해도를 높이기 위해 dinitrate로 만들었다. 새로이 합성한 [Pt (II)-(trans-l-dach)(DPPP)] $(NO_3)_2$ <KHPC-001>과 [Pt (II)(trans-l-dach)(DPPE)] $(NO_3)_2$ <KHPC-002>는 원소 분석, IR 및 ^{13}C-NMR 분석 data에 의하여 위의 물질임이 확인되었다.

KHPC-001과 KHPC-002는 MTT assay method에 의한 항암활성 연구를 통하여 P-388, L-1210 lymphocytic leukemia cell에서 항암효과가 인정되었으며, 이 항암효과는 대조 약물로 사용된 cisplatin에 비하여 우수하였다. KHPC-001과 KHPC-002는 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) complex는 carrier ligand와 leaving group의 선택에 따라 항암활성의 증가와 신독성의 감소를 일으키는 요인으로 보여지며, 이 연구에서 만들어진 두 Pt (II) complex는 앞으로 다각적인 검토를 거쳐 새로운 anticancer chemotherapeutic agent로 개발될 가능성이 있을 것으로 생각된다.