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In Vitro Antitumor Activity and Nephrotoxicity of the Novel Platinum(II) Coordination Complex Containing Cis-dach/Diphosphine

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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 nephrotoxicity.

Our platinum-based drug discovery program is aimed at developing drugs capable of diminishing toxicity and broadening the clinical spectrum of activity of cisplatin. We synthesized new Pt (II) complex analogue containing 1,2-diaminocyclohexane (dach) as carrier ligand and 1,3-bis(diphenyl phosphino)propane (DPPP) as a leaving group. Furthermore, nitrate was added to improve the solubility. A new series of PC-1 [Pt(cis-dach) (DPPP)].2NO_3 was synthesized and characterized by their elemental analysis and by various spectroscopic techniques [infrared (IR), ^{13}carbon nuclear magnetic resonance (NMR)].

PC-1 was demonstrated acceptable antitumor activity aganist SKOV-3, OVCAR-3 human ovarian adenocarcinoma ceils and significant activity as compared with that of cisplatin.

The toxicity of PC-1 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: Antitumor activity, Nephrotoxicity, Platinum coordination complex, Human ovarian adenocarcinoma

INTRODUCTION

The introduction of the square-planar complex cisplatin [cis-diaminedichloro-platinum(II)] into the clinical treatment of cancer has resulted in dramatic improvements in the response rate for some tumor types, notably testicular tumor and ovarian carcinoma (Rosenberg, 1985). While the unfavorable toxicity profile of cisplatin (primary nephrotoxicity) has been overcomed by the development of the second-generation agent, carboplatin (Calvert *et al.*, 1985; Wiltshaw, 1985; Harrap, 1985), there remains an unquestionable need for further platinum drugs which circumvent resistance. As with other cancer chemotherapeutic agents, cellular resistance to the clinically used platinum agents, cisplatin and carboplatin, represents a major clinical

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limitation to their efficacy (Loehrer and Einhorn, 1984; Mangioni *et al.*, 1989; Perren *et al.*, 1989). 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; Jacobs *et al.*, 1980; Litterest *et al.*, 1977).

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 (Cleare and Hoeschele 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 antitumor activity of Pt (II) complexes is closely correlated with the replaced rate of leaving group in vivo. (Sherman and Lippard, 1987).

To date, there has been a notable investigation for novel platinum chemistry adressing stability, broad antitumor activity, and lower nephrotoxicity. The antitumor activity of cisplatin complexes containing dach carrier ligand was investigated by Connors (1972), Cleare (1973), and Gale (1974). Kidani (1985) synthesized Pt (oxalato)(trans-1-dach)[1-OHP] and Pt(malonato)(trans-1-dach)[1-PHM] using oxalic acid/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)(cis-dach)(DPPP)].$(NO_3)_2$.

The present study reports on the synthesis of a new platinum (II) coordination complex and its antitumor activity and nephrotoxicity were evaluated with SKOV-3 and OVCAR-3 human ovarian adenocarcinoma cell lines, and 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 agent; 1,3-bis(diphenylphosphino)propane (DPPP) and cis-1,2-diaminocyclohexane (dach) were obtained from the Tokyo chemicals (JAPAN). The chemical structure of platinum analogue was presented: PC-1:[Pt(cis-dach)(DPPP)].$2NO_3$

Hormones, transferrin, and other chemicals were purchased from Sigma Chemical Corp. (St. Louis, MO, USA). Cell culture supplies (powdered medium, EDTA/trypsin and soybean trypsin inhibitor) were from Life Technologies (Grand Island, NY, USA). Class IV collagenase was obtained 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 phosphate buffered saline (PBS) prior to use.

Methods

Cell culture

Ovarian adenocarcinoma cell cultures: SKOV-3 and OVCAR-3 human ovarian adenocarcinoma cells were obtained from Cancer Reseach Center, 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 kidney proximal tubule cell cultures: Primary rabbit kidney proximal tubule 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 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 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 kidney cortical cell culture: Normal kidney tissue was freshly excised from patient undergoing radical nephrectomy in patient with renal cell carcinoma. 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 (1000rpm for 5 min) and the particles of kidney cortical tissue were suspended with DME/F12 medium supplemented with insulin (0.5 μg/ml), transferrin (5 μg/ml), hydrocortisone (5×10^{-8}M), triiodothyronine (5 ug/ml), prostaglandin E1(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.

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 plate. Three mililiters of Eagle's minimal essential medium (MEM)(GIBCO,Grand Island, NY, 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 incubater 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 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, 000rpm 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-complex was 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) was 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 reader. 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 were then produced by centrifugation (1000rpm, 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, 15, 50, 150 and 300 μM) and cultures were incubated for 48hrs 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 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 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 0.5 M-NaOH for 2 hr at 37℃. The amount of radioactivity present was determined by neutralizing with 0.5 M HCl, adding scintillating cocktail (Scint-AXF, Packard, CT, USA) and counting in a β-counter (Beckman LS 5000TD, USA).

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, MO, USA).

Measurement 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 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 vs 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

Antitumor Activity

Antitumor activity determination for cisplatin, PC-1 aganist two human ovarian adenocarcinoma cell lines is shown using MTT assay.

Fig. 1 shows the result obtained after exposure of 5, 15, 50, 150 and 300 μM aganist SKOV-3 human ovarian adenocarcinoma cell-line. PC-1 showed concentration-dependent increase in antitumor activity, and cytotoxicity of PC-1 showed only 24.2% and 37.1% of CI at 5 μ

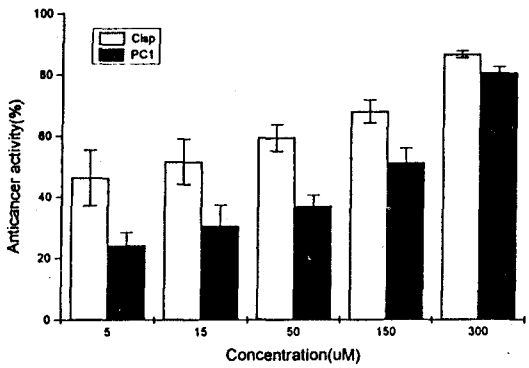

Fig. 1. Anticancer activities of platinum(II) complexes on the SKOV-3 human ovarian adenocarcinoma cells.
Cisp: Cisplatin
PC1: [Pt(II)(cis-dach)(DPPP)]2NO_2

M and 50 μM, respectively aganist cells, and then antitumor activity was only acceptable at 150 M and 300 μM (CI: 56.5/86.8%).

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

NEPHROTOXICITY

Rabbit kidney proximal tubule cells

The cytotoxicities of cisplatin and PC-1 aganist rabbit kidney proximal tubular cells as determination by MTT assay are shown in Fig 3. PC-1 (CI: 5.0%) showed less cytotoxic at 5 μ M as compared with that of cisplatin (CI: 39.2 %). At a concentration of 50 μM and 300 μM, PC-1 showed 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 1. PC-1 showed 48.1% of [^{3}H]-thymidine uptake as compared with that of cisplatin (1.5%) at 500 μ M. This result indicates that cytotoxicity of PC-1 was significantly less than that of cisplatin and [^{3}H]-thymidine uptake assay is more sensitive than MTT test (up to 7-fold).

Human kidney cortical cells

PC-1 showed less cytotoxicity (CI: 10.4%) as compared with that of cisplatin (CI: 70.2%) at 50 μM (Fig.4).

Table 2 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 PC-1 (43.6 %) and PC-2 (42.3%).

Table 1. Effect of Pt(II) complexes on ^{3}H-thymidine incorporatin into primary cultured proximal tubular cells of rabbit kidney

Group	^{3}H-thymidine uptake (cpm/105cells)	Uptake rate(%)
Contro	598.3±75.15	100.0
Cisplatin	9.0± 3.46	1.5
PC-1	288.0±59.01	48.1

Concentration of Pt(II)-complexes in culture medium: 5×10^{-5} M

PC-1: [Pt(cis-dach)(DPPP)]$(NO_3)_2$

Values are means±S.E. All the incorporations were determined in triplicate

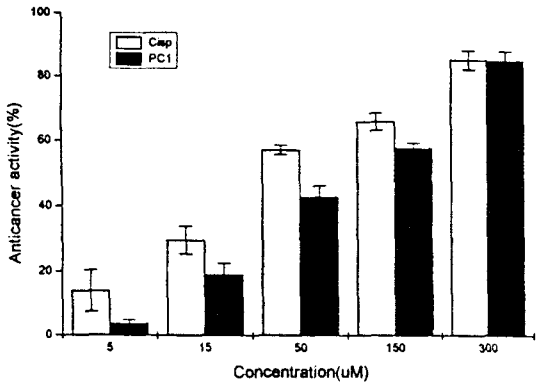

Fig. 2. Anticancer activities of platinum(II) complexes on the OVCAR-3 human ovarian adenocarcinoma cells.
Cisp: Cisplatin
PC1: [Pt(II)(cis-dach)(DPPP)]2NO_3

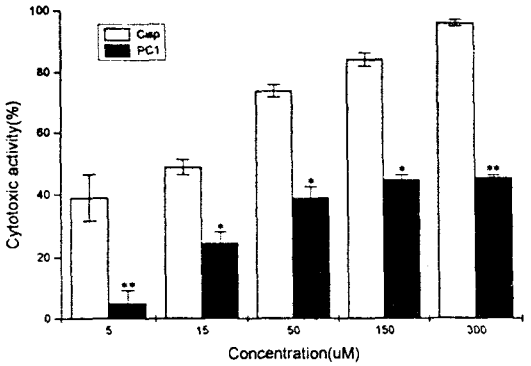

Fig. 3. Cytotoxic activities of platinum(II) complexes on the proximal tubule cells of the rabbit kidney.
Cisp: Cisplatin
PC1: [Pt(II)(cis-dach)(DPPP)]2NO_3

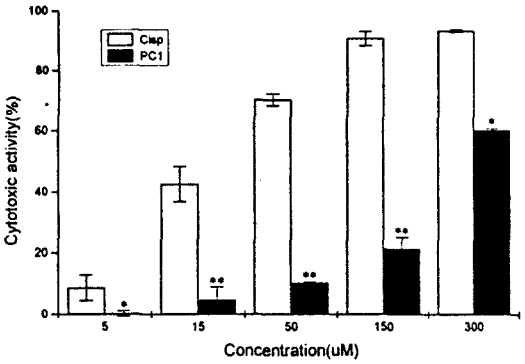

Fig. 4. Cytotoxic activities of platinum(II) complexes on the renal cortical cells of human kidney.
Cisp: Cisplatin
PC1: [Pt(II)(cis-dach)(DPPP)]2NO_3

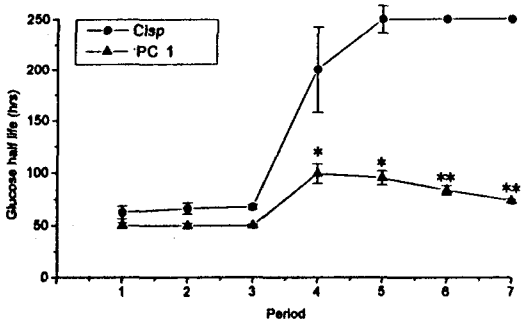

Fig. 5. Nephrotoxicity on 3 weeks histocultured human kidney.
Toxicity was measured by glucose consumption. Each drug exposed for 72hrs with 50 uM in concentration.
Cisp: Cisplation
PC-1: [Pt(cis-dach)(DPPP)]-2NO_3
*dach: 1,2-Diaminocyclohexane
DPPP: 1,3-Bis(diphenylphosphino)propane

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 signif-

Table 2. Effect of Pt(II) complexes on ^{3}H-thymidine incorporatin into primary cultured renal cortical cells of human kidney

Group	^{3}H-thymidine uptake (cpm/105cells)	Uptake rate(%)
Contro	621.3±56.01	100.0
Cisplatin	8.7± 5.14	1.4
PC-1	271.0±41.28	43.6

Concentration of Pt(II)-complexes in culture medium: 5×10^{-5} M
PC-1: [Pt(cis-dach)(DPPP)]$(NO_3)_2$
Values are means±S.E. All the incorporations were determined in triplicate

icance.

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 PC-1 was less marked than cisplatin(Fig. 5).

DISCUSSION

The platinum coordination complexes are cytotoxic agents that were first identified by Rosenberg *et al.*, (1965). The inhibitory effects on bacterial replication were subsequently shown to be due to the formation of inorganic platinum-containing compounds in the presence of ammonium and chloride ions. cis-Diaminedichloroplatinum (II) (cisplatin) was found to be the most active 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 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 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.*, 1977). 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 into 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 (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 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 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 antitumor activity and nephroroxicity using several cancer cell lines (SKOV-3 and NIH-OVCAR-3 human ovarian adenocarcinoma cells) and human/ rabbit kidney cells, respectively. New synthetic Pt(II)-complex, PC-1 exhibited significant antitumor activity aganist human ovarian adenocatcinoma cell lines.

A criteria for antitumor activity in vitro is generally expressed in cytotoxicity index in SKOV-3 and OVCAR-3 and more than 50% in cytotoxicity index is accepted as positive antitumor drugs. The new Pt (II) complex showed comparable antitumor activity to cisplatin.

The results obtained here presented that new platinum-complex compound had less cytotoxic than cisplatin. This is conceivable that modification of the carrier ligand as a diaminocyclohexane and leaving group as a DPPP 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-PK1 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 con-

sumption 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 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)(cis-dach)(DPPP)].$(NO_3)_2$의 항암효과 및 신독성

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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 diphosphine류인 1,3-bis (diphenylphosphine의 propane(DPPP) 을 도입하였으며, 물에 대한 용해도를 높이기 위해 dinitrate로 만들었다. 새로이 합성한 [Pt(II)(cis-dach)(DPPP)].$(NO_3)_2$ <PC-1>은 원소 분석, IR 및 ^{13}C-NMR 분석 data에 의하여 위의 물질임이 확인되었다.

PC-1은 MTT assay method에 의한 항암활성 연구를 통하여 SKOV-3, OVCAR-3 human ovarian adenocarcinoma cells에서 항암효과가 인정되었으며, 이 항암효과는 대조 약물로 사용된 cisplatin과 유사하였다.

PC-1은 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 새로운 Pt(II) complex는 앞으로 다각적인 검토를 거쳐 새로운 anticancer chemotherapeutic agent로 개발될 가능성이 있을 것으로 생각된다.