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Cytotoxicity of Two Novel Cisplatin Analogues, $(CPA)_2Pt$[DOLYM] and (DACH)Pt[DOLYM], to Human Cancer Cells *In Vitro*

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Despite the impressive antitumor activity of cisplatin, two major limitations of the drug, that is severe side effects and drug-resistance of cancer cells, make its use difficult for cancer therapy. These limitations have resulted in a great deal of effort having been expended into structural modifications of cisplatin. In this study, we tested two novel cisplatin analogues, $(CPA)_2Pt$ [DOLYM] (COMP-I) and (DACH)Pt[DOLYM] (COMP-II), for the mode of cytotoxic action against human tumor cells comparing with cisplatin and carboplatin *in vitro*. These two novel analogues had considerable cytotoxic activities against five kinds of human solid tumor cells, and especially COMP-II was more effective on HCT15 colon cancer cells than other compounds. In addition, COMP-II had cytostatic activity at low concentrations (10~0.3 μg/ml), but other compounds revealed little effect on tumor growth at the low concentration.

Key words : Cisplatin, Cytotoxicity, Human cancer cells

INTRODUCTION

Cisplatin, one of the clinically most useful anticancer agents, is used alone or in combination with other agents against a wide variety of tumor types such as breast (Sledge *et al.*, 1992), head and neck (Forastiere, 1992), urothelial tract (Sternberg *et al.*, 1993), bladder (Sternberg *et al.*, 1993a), lung (Sandler *et al.*, 1992), ovary and testes (Prestayko *et al.*, 1979), stomach (Wils, 1991) and etc. However, the appearance of several side effects and drug-resistant cancer cells limits its use in cancer clinic (Prestayko *et al.*, 1979.; Andrew *et al.*, 1990.; Perez *et al.*, 1991). These limitations have spurred the development of new platinum analogues.

The common way of administering platinum compounds to patients is by intravenous infusion. Infusion times vary from a few minutes up to 24 h or longer. Platinum concentrations in malignancies 24 h or more after administration of the platinum drug might be considered as a measure of anti-tumor activity (Calvert *et al.*, 1993). Therefore, the pharmacokinetic properties of a platinum compound are very important factor in cancer clinic.

We have already reported the anticancer activity of several novel cisplatin analogues to mouse tumor cell line, L1210 (Sohn *et al.*, 1994). In the previous report, we have shown that all of the analogues tested have anticancer activity comparable with that of cisplatin or at least with that of carboplatin *in vitro*, and some of them have significant effect *in vivo*. In this experiment, we have selected two analogues, i. e. $(CPA)_2Pt$[DOLYM] (COMP-I) and (DACH)Pt[DOLYM] (COMP-II), based on the activity *in vitro* and *in vivo* in order to gain more informations for human tumors. The present report describes the cytotoxic effects of these two analogues to five kinds of human solid tumor cell lines and the effects of drug exposed time and cell growth recovery *in vitro*.

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MATERIALS AND METHODS

Chemicals

COMP-I and COMP-II were synthesized chemically in Korea Institute of Science & Technology (Seoul, Korea), and detailed chemical procedures were described on the previous paper (Sohn *et al.*, 1994). Cisplatin and carboplatin were purchased from Sigma Chemical Company (St. Louis, MO, USA). The structures of platinum complexes tested in this study were shown in Fig 1. RPMI 1640 cell growth medium, trypsin and fetal bovine serum (FBS) were obtained

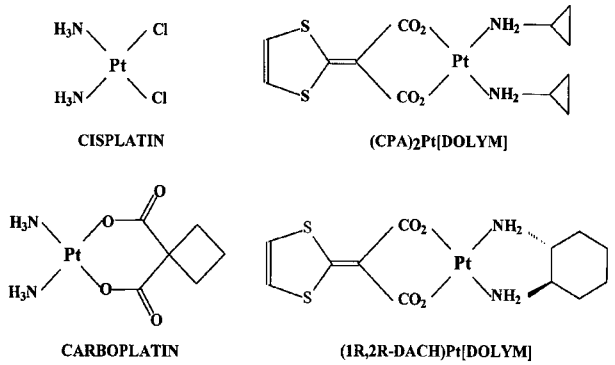

Fig. 1. Structures of the four platinum complexes used in this study.

from Gibco Ltd. (Grand Island, N. Y., USA). Other cell culture agents such as gentamycin, amphotericin, 12-cyclohexanediaminetetraacetic acid (CDTA), sodium bicarbonate, and agents for cytotoxicity test such as trichloroacetic acid (TCA), sulforhodamine B (SRB) and trisma base were purchased from Sigma Chemical Company.

Cells

All the cell lines used in this study were of human origin. These include the lung carcinoma cell line (A549), the colon adenocarcinoma cell line (HCT15), the ovary adenocarcinoma cell line (SK-OV-3), the malignant melanoma cell line (SK-MEL-2) and the central nerve system carcinoma cell line (XF498). Stock cell cultures were grown in T-25 (Falcon) flasks containing 10 ml of RPMI 1640 medium with glutamine, sodium bicarbonate, gentamycin, amphotericin and 5% FBS. The cells were dissociated with 0.25% trypsin and 3 mM CDTA solution in cases of transferring or dispensing before experiment. The cells were maintained in the incubator at 37°C in a humidified atmosphere of 5% CO_2 in air continuously except when adding drugs.

Cytotoxicity studies

The cytotoxicity studies were carried out with the SRB microculture colorimetric assay as described previously (Skehan *et al.*, 1990., Ryu *et al.*, 1992). Briefly, cells were plated into 96-well flat bottom plates on day 0 and exposed to drug on day 1. The SRB assay was carried out 48 hours after drug exposure. At the termination of incubation with each drug at various concentrations, the cells were fixed with 10% TCA *in situ*, washed and dried. Then, 0.4% SRB solution in 1% acetic acid was added and incubated at room temperature. The cells were washed again and the bound stain was solubilized with trisma base solution (pH 10.2~10.5), and measured spectrophotometrically at 520 nm and 690 nm in a microtiter plate reader. The absorbance measured at 690 nm was subtracted from the absorbance at 520 nm so as to eliminate the effects of non-specific absorbance.

The data were transferred and transformed into Microsoft Excel format and survival fraction at each concentration of platinum complex was calculated by comparing with control. All data represented the average values of three wells.

Cytokinetic studies *in vitro*

HCT15 cells were seeded in 96-well flat bottom plate, and the cells were preincubated for 24 hours. After preincubation, the test-drug solutions were added to the cultures and incubated for various time (1, 3, 6, 9, 12, 18, 24, 36, 48, 60 hours). After drug exposed time, the cells were fixed by TCA solution *in situ*, and the cytotoxicity was measured by SRB assay.

Cell growth recovery studies

All experimental procedures were same with those of cytokinetic study except fixing *in situ* after drug exposed time. At the termination of drug exposed time, the drug containing medium was removed immediately by aspiration and the cultures were washed twice with fresh medium (without drug). Then, new fresh medium was added to the culture and incubated up to 60 hours including drug exposed time. After cell growth recovery time, the cell survival fractions were measured by SRB assay.

RESULTS AND DISCUSSIONS

Cytotoxicity to cancer cells

Since the early work in the preclinical and clinical development of cisplatin, lots of analogues have been synthesized and tested for the properties that would enhance the therapeutic index of cisplatin specifically with the hope of overcoming platinum resistance or reducing toxicities. More than 13 of these analogues have been evaluated in clinical trials so far, but no one has provided definite advantage over cisplatin and achieved worldwide approval, except carboplatin (Hamilton *et al.*, 1993; Weiss and Christian, 1993). Therefore, analogues of cisplatin are still synthesized and studied preclinically by many laboratories, and already we have also reported several analogues for antitumor activities against mouse cancer cell line *in vitro* and *in vivo* (Shon *et al.*, 1994). In this study, we tested two novel cisplatin analogues for antitumor activity against human solid tumor cells in comparison with cisplatin and carboplatin *in vitro*. The concentrations that cause 50% cell growth inhibition (IC_{50}) of cisplatin to A549, SK-OV-3, SK-MEL-2, XF498 and HCT15 cells were 1.24, 0.83, 0.79, 0.88 and 2.13 μg/

ml, respectively. The IC_{50}s of carboplatin to those cells were 15-25 fold higher than those of cisplatin. The IC_{50}s of COMP-I to those cells were 1.5-2 fold higher than those of cisplatin, except to A549 (1.23 μg/ml). Meanwhile, the IC_{50}s of COMP-II to those cells were 0.27, 2.04, 0.90, 1.94 and 0.66 μg/ml, demonstrating that A549 and HCT15 cells were relatively sensitive to COMP-II in comparison with other compounds tested. The IC_{50}s of the compounds tested were summarized in Table I.

Among the cell lines tested, HCT15 colon carcinoma cells were the most resistant to cisplatin, carboplatin and COMP-I. Meanwhile, the HCT15 cells were relatively sensitive to COMP-II, which has including the moiety of diaminocyclohexane (DACH) in comparison with other cells tested. In a previous report, oxaliplatin, a cisplatin analogue contained DACH moiety, also revealed a good cytotoxicity to HT-29 human colon cancer cells in comparison with other cells such as ovarian cancer (A2780 and A2780/cp), bladder cancer (TCCSUP and RT4), melanoma (SK-MEL-2 and HTB144) and glioma (U373MG and U87MG) (Pendyalla and Creaven, 1993). In this study, A549 cells were also sensitive to COMP-II in comparison with other compounds tested. These two cells, i. e. A549 and HCT15 cells, are Ki4B-Ras mutated cells, and grow fast than other cells tested.

Kinetics *in vitro*

The effects of drug exposed time (cytokinetic) and cell growth recovery were shown in Fig. 2 (carboplatin), Fig. 3 (cisplatin), Fig. 4 (COMP-I) and Fig. 5 (COMP-II). Cell growth recovery was not shown in all the cases of compounds tested. Carboplatin had cytotoxic activity at the concentrations of 300 and 100 μg/ml, and it revealed cytostatic activity at 30 μg/ml. There was no visible effect up to 12 hours after carboplatin exposure at all concentrations tested. In cisplatin and COMP-I, they need 9 hours at high concentration (100 μg/ml) for showing more than 50% inhibitory effect, and COMP-II was faster than cisplatin and COMP-I. Cisplatin and COMP-I had cytotoxic activity at 100 and 30 μg/ml. Meanwhile, at 10 μg/ml, COMP-I

Table I. Anticancer activities of some platinum complexes against human tumor cell lines *in vitro*

Compound	IC_{50} (μg/ml)[a]				
	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
Carboplatin	24.27±4.05[b]	15.78±5.13	14.16±5.51	20.16±2.29	50.67±7.43
Cisplatin	1.24±0.11	0.83±0.17	0.79±0.28	0.88±0.16	2.13±0.25
$(CPA)_2Pt$[DOLYM]	1.23±0.34	1.98±0.51	1.36±0.43	1.41±0.23	2.80±0.68
(DACH)Pt[DOLYM]	0.27±0.13	2.04±0.55	0.90±0.38	1.94±0.31	0.66±0.27

[a]IC_{50} value of compound against each cancer cell line, which was defined as concentration that caused 50% inhibition.
[b]Data are Mean±S.E. of at least four distinct experiments.

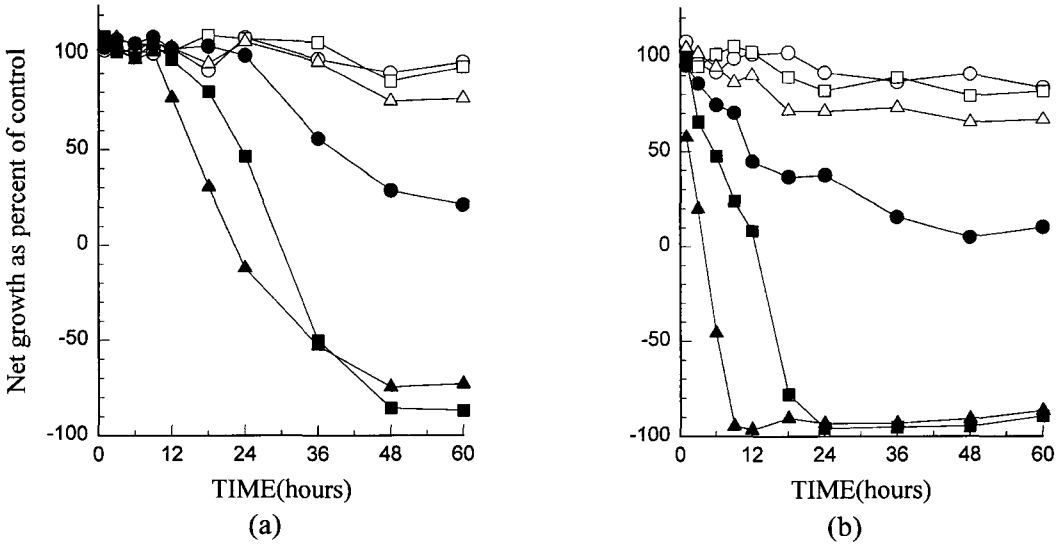

Fig. 2. Effects of drug exposed time (a) and cell growth recovery (b) of carboplatin on HCT15 human colon cancer cells. The carboplatin solutions at different concentrations were added to the cultures at time 0 and incubated for various time. After drug exposed time, the cells were fixed *in situ* (a) or incubated up to 60 hours after washing and replacing with fresh medium (b). Concentrations (μg/ml); 1 (○), 3 (□), 10 (△), 30 (●), 100 (■), 300 (▲).

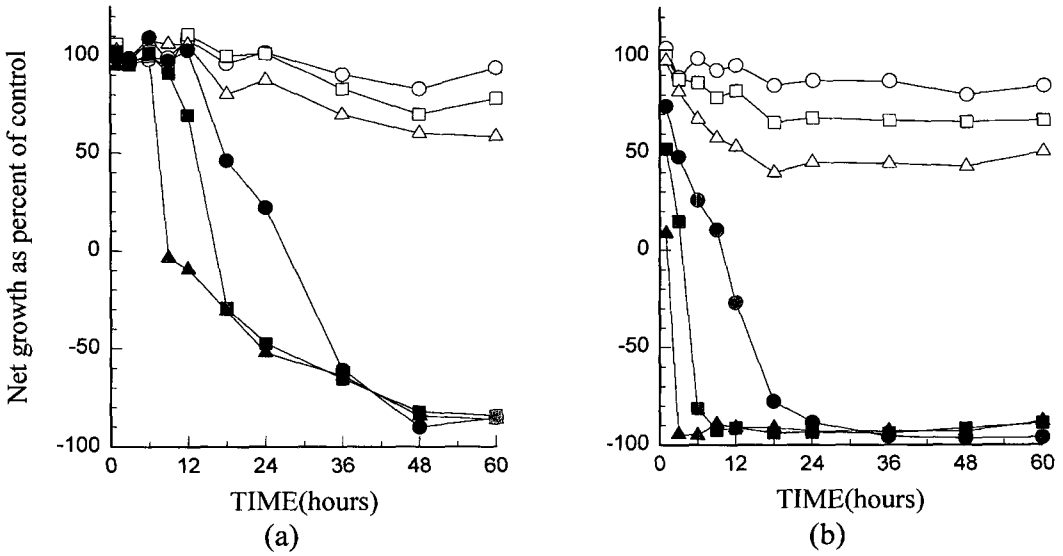

Fig. 3. Effects of drug exposed time (a) and cell growth recovery (b) of cisplatin against HCT15 human colon cancer cells. The cisplatin solutions at different concentrations were added to the cultures at time 0 and incubated for various time. After drug exposed time, the cells were fixed *in situ* (a) or incubated up to 60 hours after washing and replacing with fresh medium (b). Concentrations (μg/ml); 0.3 (○), 1 (□), 3 (△), 10 (●), 30 (■), 100 (▲).

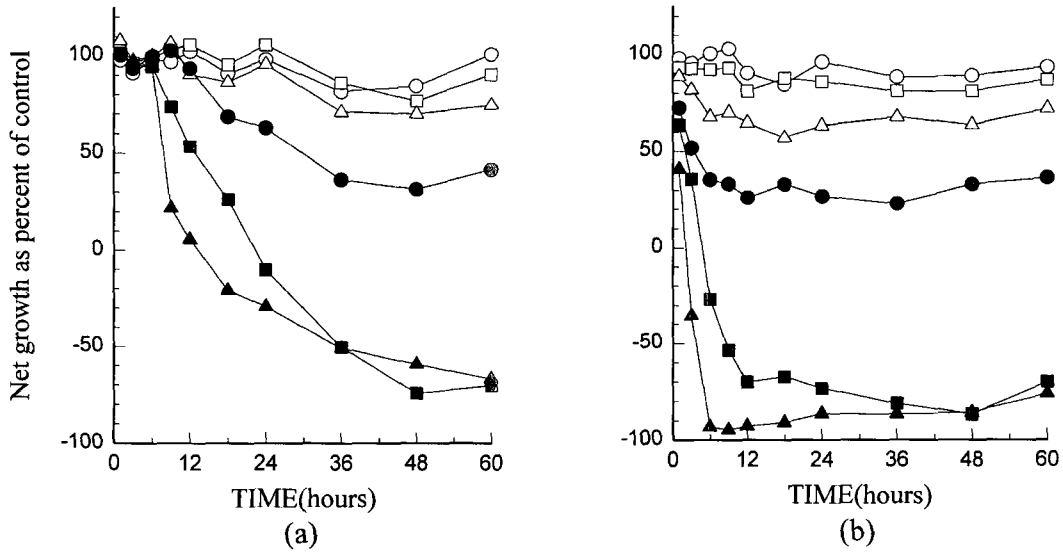

Fig. 4. Effects of drug exposed time (a) and cell growth recovery (b) of $(CPA)_2Pt[DOLYM]$ on HCT15 human colon cancer cells. The $(CPA)_2Pt[DOLYM]$ solutions at different concentrations were added to the cultures at time 0 and incubated for various time. After drug exposed time, the cells were fixed *in situ* (a) or incubated up to 60 hours after washing and replacing with fresh medium (b). Concentrations (μg/ml); 0.3 (○), 1 (□), 3 (△), 10 (●), 30 (■), 100 (▲).

revealed cytostatic activity, while cisplatin revealed cytotoxic activity. In the presence of carboplatin and COMP-I, the cytotoxic mode of drug exposed time and cell growth recovery against HCT15 cells were similar, and that of cisplatin was different a little from these compounds. Meanwhile, COMP-II had something more different from those patterns to the other compounds tested. COMP-II was cytotoxic to HCT15 cells at the concentration of 100 μg/ml, and it had mild cytotoxicity at 30 μg/ml. At relatively low concentrations (0.3~10 μg/ml) COMP-II revealed cytostatic activity rather than cytotoxic activity.

In pulse duration assay, 1 h exposure of HCT15 cells to cisplatin and COMP-I at 100 μg/ml reduced the cell growth approximately 90 and 40%, respectively. By contrast, in COMP-II at 100 μg/ml, HCT15 cells were killed approximately 30% in comparison with starting control. At 30 μg/ml for 1 h, cisplatin, COMP-I, and COMP-II reduced the HCT15 cell growth approximately 50, 40 and 70%. Among the platinum complexes tested, COMP-II revealed a good cytostatic activity at the low concentrations. The effect of COMP-

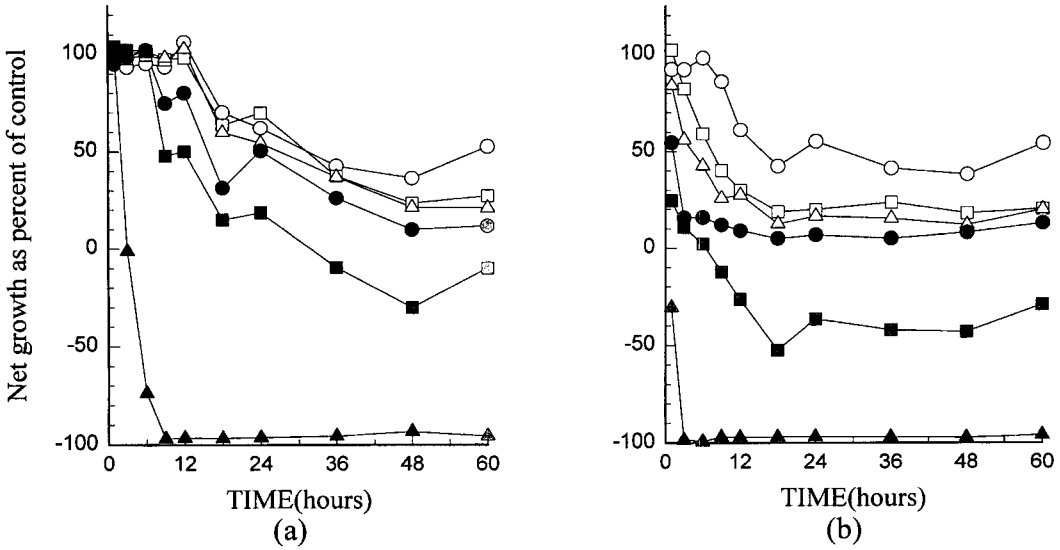

Fig. 5. Effects of drug exposed time (a) and cell growth recovery (b) of (DACH)Pt[DOLYM] on HCT15 human colon cancer cells. The (DACH)Pt[DOLYM] solutions at different concentrations were added to the cultures at time 0 and incubated for various time. After drug exposed time, the cells were fixed *in situ* (a) or incubated up to 60 hours after washing and replacing with fresh medium (b). Concentrations (μg/ml); 0.3 (○), 1 (□), 3 (△), 10 (●), 30 (■), 100 (▲).

II at 0.3 μg/ml was similar to that of cisplatin at 10-fold higher concentration.

The concentration of a drug in the tumor-bearing organ is decreased after reaching maximal concentration. Accordingly, the time required to achieve a effect of a drug at a concentration is a critical factor in cancer clinic. The effectiveness of a drug at a relatively low concentration is also a important factor in the clinic. In this study, COMP-II had a relatively wide-range active concentration, and its activity pattern was mainly cytostatic rather than cytotoxic. From these results, accordingly, we suspect that COMP-II has a good pharmacokinetic property in comparison with other compounds tested.

In deed, in these limited size of tumor cell panel, it is difficult to make generalized perspectives about the *in vivo* results with the reference of *in vitro* activities of drugs. Nonetheless, this study revealed that COMP-II showed remarkable activity against human cancer cell lines, and it shall be meaningful to study more analogues containing (DACH) ligand.

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