Short Communication

Analysis of Cisplatin Behavior in a Non-Small Cell Lung Cancer (NSCLC) Cell Line

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Abstract

Cisplatin (CDDP) is an effective anticancer agent that is widely used in the treatment of testicular, ovarian, bladder and lung cancers. However the development of resistance to CDDP by tumor cells is a major obstacle to treatment. We reported that decreased accumulation of CDDP was observed in CDDP-resistant cell lines from NSCLC, and a good correlation was found between the amount of intracellular platinum and the sensitivity of lung cancer cell lines to CDDP. In the present study, to investigate the CDDP resistance mechanism, several platinum compounds were exposed to those cell lines, and we measured the cellular platinum using inductively coupled plasma mass spectrometry (ICP-MS). Furthermore, the proportion of intact CDDP in total platinum was also determined by LC-ICP-MS for pharmacokinetic study of CDDP.

Keywords : cisplatin resistance, NSCLC, intracellular Pt accumulation, Pt-DNA adduct, intact CDDP

Introduction

The major problem in cancer chemotherapy is the emergence of inherent and acquired drug resistance of the cancer cells. Although many studies have been done to investigate the mechanism of CDDP resistance, the precise nature of the resistance in cancer cells is still unclear. The molecular mechanism of CDDP resistance has been revealed to be "multifunctional"; it decreased CDDP accumulation, increased intracellular detoxification and increased the DNA repair ability [1]. Previously, we established CDDP-resistant cell lines and characterized them to elucidate the resistance mechanism. A decrease in platinum accumulation was observed in resistant cell lines to CDDP [2]. There was also a good correlation between the intracellular amount of platinum and sensitivity against CDDP. This result suggests that the intracellular accumulation of platinum is a major determinant of the CDDP resistance, at least in non-small cell lung cancer (NSCLC). Then, a key question is why the uptake of CDDP is suppressed in the resistant cell line. The accumulation of platinum was compared with parental and resistant cell line using CDDP and its closely related platinum compounds. Furthermore, the proportion of intact CDDP in total platinum was also determined by LC-ICP-MS for pharmacokinetic study of CDDP.

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Materials and Methods

We used a human NSCLC cell line, PC-9, and its CDDP-resistant subline, PC-9/CP^r. These cells were maintained in RPMI1640 medium containing 10% fetal bovine serum, 2mM L-glutamine, 100U/mL penicillin, 100μg/mL streptomycin, using 75cm^2 flasks in a water-saturated atmosphere (95% air / 5% CO_2) at 37℃.

Drug-sensitivity was determined using MTT (3-[4,5-

dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay, and quantified by measuring the absorbance of converted dye at 570nm with background subtraction at 655nm. The percentage of growth inhibition was plotted against concentration of the platinum compounds in medium.

^{199}Pt tracer solution was obtained from the Au target irradiated in the RIKEN Ring Cyclotron. For the uptake experiments, the PC-9 and PC-9/CP^r were exposed to the ^{199}Pt tracer. Further, several non-radioactive platinum compounds (CDDP, transplatin, carboplatin, nedaplatin, *cis*-dichloro [ethylenediamine]platinum [*cis*-DEP], hydrogen hexachloro platinate[H_2PtCl_6]) were also exposed to both cell lines. After incubation, radioactivity was measured using a Ge detector, and total platinum was determined by ICP-MS. The intact CDDP was measured by using LC-ICP-MS (Perkin Elmer) under the condition shown in Table 1. Under these conditions, intact CDDP was eluted at the retention time of 7.3min (Fig. 1).

Results and Discussion

In the drug-sensitivity assay, CDDP resistant cell line PC-9/CP^r showed about 10-fold higher CDDP resistance than parental PC-9. Cross-resistance was observed to carboplatin, nedaplatin, and *cis*-DEP, but not to transplatin and H_2PtCl_6 (Table 2). The accumulation of ^{199}Pt tracer in CDDP-resistant cell line was about 60% of that in parental cells. On the intracellular platinum accumulation using platinum compounds, CDDP, carboplatin, nedaplatin, and *cis*-DEP were decreased in PC-9/CP^r (Fig. 2).

The relationship between the structure and uptake of platinum compounds has discussed, and *cis*-geometry of amines in platinum compounds is essential for the drug resistance.

We also measured the amount of DNA adduct of these platinum compounds. The anticancer activity of CDDP is attributed to its ability to form Pt-DNA adducts. After exposure of 10μM of each platinum compounds to both cell lines for 24hr, cells were harvested, and genomic DNA was extracted and measured the content of platinum using ICP-MS.

The amount of Pt-DNA adducts of CDDP, carboplatin, nedaplatin, and *cis*-DEP has decreased in PC-9/CP^r as well as the intracellular platinum accumulation. The ratio of DNA adduct of these platinum compounds to the intracellular platinum accumulation was about 2.5%, and there was no significant difference in parental and resistant cell lines. These results suggest that the amount of Pt -DNA adducts depends on the intracellular platinum con-

Table 1 LC-ICP-MS conditions

HPLC conditions		
HPLC column	:	TSKgel Amide-80 2.0×150mm (TOSOH), 30℃
Mobile phase	:	MeOH : H_2O = 95 : 5, 0.2mL/min
ICP-MS operating conditions		
ICP-MS	:	Elan DRC II (Perkin Elmer)
Nebulizer gas flow	:	1.07L/min
Aux. gas flow	:	1.10L/min
Plasma gas flow	:	17.00L/min
Ion-lens voltage	:	5.25V
ICP RF power	:	1500W
Scan mode	:	Peak hopping
Isotope measured	:	^{195}Pt

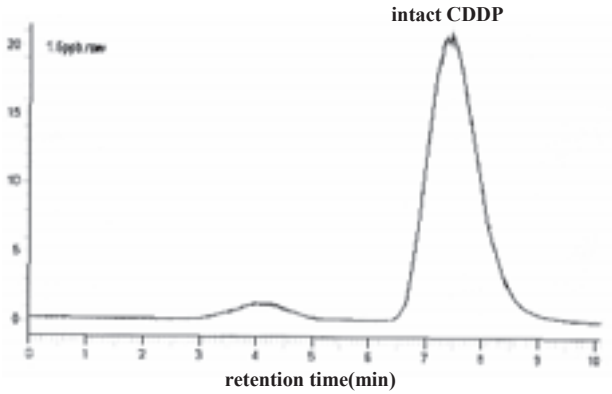

Fig. 1 Chromatogram of intact CDDP

Table 2 Sensitivity to Pt compounds after 72hr exposure

Pt compounds	IC_{50}(μ M)		Relative sensitivity*
	PC-9	PC-9/CP^r	
CDDP	1.13±0.12	11.2±0.76	9.91
transplatin	85.3±5.03	108.7±9.87	1.27
carboplatin	13.3±3.21	106.7±15.3	8.02
nedaplatin	4.03±2.51	48.7±2.31	12.1
cis-DEP	5.77±1.97	15.3±0.58	2.65
H_2PtCl_6	20.0±1.73	20.3±0.58	1.02

* Relative sensitivity was calculated as IC_{50}[PC-9/CP^r]/[PC-9].

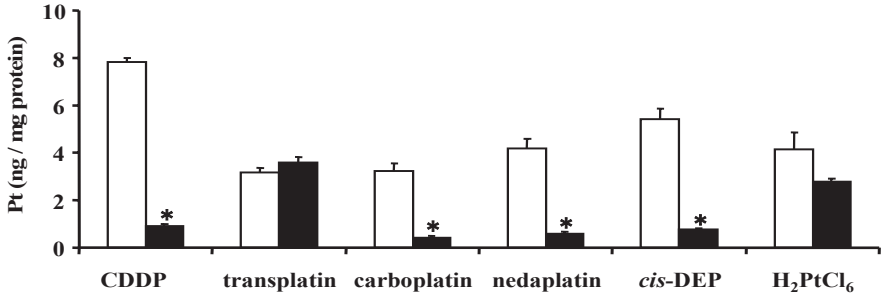

Fig. 2 Accumulation of Pt in PC-9 and PC-9/CP^r after 4hr of exposure to 2μM Pt compounds
Each bar represents the mean of three independent experiments. *Vertical bars*, ±SD. $^*P<0.01$.
□ : PC-9, ■ : PC-9/CP^r.

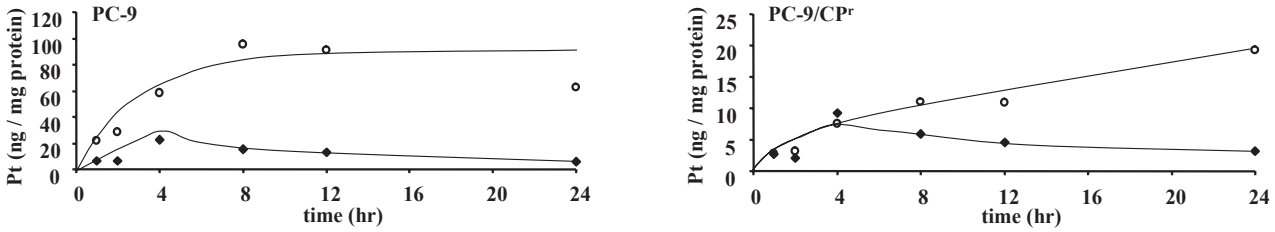

Fig. 3 Time-courses of total Pt and intact CDDP in cells after exposure to 10μM CDDP for various time intervals
○ : total Pt, ◆ : intact CDDP.

centration.

Furthermore, the proportion of intact CDDP in total platinum was also determined in both cell lines, by using LC-ICP-MS under the condition shown in Table 1. As shown in Fig. 3, the change of the intracellular platinum concentration was different in both cell lines. The total platinum accumulation in the cells reached the maximum in PC-9 after 8hr exposure, but increased continuously until 24hr in the case of PC-9/CP^r. On the other hand, intact CDDP in the cells has decreased gradually after 4hr exposure of the CDDP in both PC-9 and PC-9/CP^r. Almost intracellular Pt existed as the intact CDDP in PC-9/CP^r, while only 40% of that existed in PC-9 at the time of 4hr CDDP exposure. As mentioned above, it can be expected that the intact CDDP is taken in the cell at the first stage of the CDDP exposure and, thereafter metabolic process of PC-9/CP^r is different from PC-9.

In the present study, we have applied a LC-ICP-MS method for the quantitative determination of intact CDDP in the CDDP-resistant cell line. The data show that the kinetics of intact CDDP has significant difference in the parental and resistant cell lines. The different content levels of intact CDDP may suggest the presence of some proteins that regulates the uptake of CDDP. CDDP is also known to be reactive against some biologically relevant low-molecular-mass compounds. The investigations to identify the metabolites of CDDP are currently under way.

References

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