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ORIGINAL ARTICLE

# Factors affecting sensitivity to antitumor platinum derivatives of human colorectal tumor cell lines

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**Abstract**
*Purpose* The aim of this study is to examine the factors affecting sensitivity to cisplatin, carboplatin, and oxaliplatin in human colorectal tumor cell lines.
*Methods* Caco-2, DLD-1, HCT-15, HCT116, LS180, SW620, and WiDr cells were used. Their growth inhibition by platinum derivatives was evaluated with a WST-1 assay utilizing succinate dehydrogenase activity. Cellular accumulation and DNA-binding of platinum were measured with an inductively coupled plasma mass spectrometer. The mRNA levels of copper transporters (hCtr1, ATP7A, and ATP7B) and organic cation transporters (hOCT1, hOCT2, and hOCT3) were evaluated by the real-time reverse transcription-PCR method using SYBR® green.
*Results* The cytotoxicity of platinum derivatives ranked oxaliplatin > cisplatin > carboplatin in almost all cells used. Cellular accumulation and DNA-binding of platinum varied among the types of cells, but levels were similar on treatment with cisplatin and oxaliplatin, and lower in response to carboplatin. The levels of copper and organic cation transporter mRNAs also differed with cell type. A correlation analysis revealed that sensitivity to platinum derivatives was dependent in part on the amount of platinum bound to DNA. In addition, the cellular accumulation of platinum and level of ATP7A mRNA may be factors affecting the cytotoxicity of cisplatin, while the cytotoxicity of oxaliplatin was suggested to be affected by the levels of ATP7A and hOCT1 mRNAs.
*Conclusion* Some factors affecting the sensitivity of tumor cells to platinum derivatives were proposed, and will provide useful information for cancer chemotherapy with platinum derivatives.

**Keywords** Cisplatin · Carboplatin · Oxaliplatin · Colorectal tumor · Drug sensitivity

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## Introduction

Platinum derivatives have been used world wide in the treatment of solid tumors in the esophagus, stomach, lung, and ovaries [1]. Cisplatin has been the most important of these drugs for several decades. Severe nephrotoxicity, however, limits the use of cisplatin even now [2]. Other platinum derivatives such as carboplatin and nedaplatin have also been prescribed for patients with solid tumors, and are considered less nephrotoxic than cisplatin [3]. In addition, a third generation platinum derivative, oxaliplatin, was clarified to have unique characteristics showing increased antitumor activity against colorectal cancer [4, 5].

The main mechanism of action for platinum derivatives is the intercalation of platinum into DNA, resulting in the inhibition of DNA synthesis [6]. Thus, the cellular kinetics of platinum is considered a significant factor determining sensitivity to platinum derivatives. As the derivatives differ in effectiveness and safety [7], the identification of factors

affecting the sensitivity of cells should help to enhance clinical outcome in cancer chemotherapy. Regrettably, little such information is available at present.

In the present study, we evaluated the sensitivity to cisplatin, carboplatin, and oxaliplatin of seven human colorectal cancer cell lines. Based on these findings, the relationship between the sensitivity of tumor cells and the factors participating in the cellular kinetics of platinum derivatives, i.e., the accumulation of platinum in cells, the binding of platinum to DNA, or the expression of transporters affecting the incorporation of platinum into cells, were analyzed.

## Materials and methods

### Chemicals

Cisplatin and carboplatin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Oxaliplatin was purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). 2-(4-Iodophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt (WST-1), 1-methoxy-5-methylphenazinium methylsulfate (1-methoxy PMS), and HEPES used in the WST-1 colorimetric assay were purchased from Dojindo Laboratories (Kumamoto, Japan).

### Cells and cell culture

Seven kinds of cell lines, Caco-2, DLD-1, HCT-15, HCT116, LS180, SW620, and WiDr, were used as a human colorectal cancer cell model. Cells were cultured in an atmosphere of 95% air and 5% $CO_2$ at 37°C, and subcultured every 3 or 4 days at a density of $2 \times 10^6$ cells/100 mm dish. The number of passages for Caco-2, DLD-1, HCT-15, HCT116, LS180, SW620, and WiDr cells was 53–66, 29–39, 58–68, 32–43, 55–64, 101–111, and 30–40, respectively.

### WST-1 colorimetric assay

#### *Growth inhibitory activity of platinum derivatives*

The growth inhibitory effects of cisplatin, carboplatin, and oxaliplatin were evaluated with a WST-1 colorimetric assay utilizing succinate dehydrogenase activity [8, 9]. Cells ($5 \times 10^3$/well) were seeded into 96-well plates (Corning Inc., NY, USA) in 100 μl of culture medium without any drugs on Day 0. The culture medium was exchanged for that containing test drugs at various concentrations on Day 1. After incubation for 72 h at 37°C (on Day 4), the culture medium was exchanged for 110 μl of that containing WST-1 solution (10 μl of WST-1 solution and 100 μl of culture medium), and 3 h later the absorbance was determined at 450 nm with a reference wavelength of 620 nm using a microplate reader (Spectra Fluor$^{TM}$, Tecan, Switzerland). The 50% growth inhibitory concentrations ($IC_{50}$) were calculated according to the sigmoid inhibitory effect model $E = E_{max} \times [1 - C^{\gamma}/(C^{\gamma} + IC_{50}^{\gamma})]$ using the nonlinear least-squares fitting method (Solver, Microsoft® Excel). $E$ and $E_{max}$ represent the surviving fraction (% of control) and its maximum, respectively, and $C$ and $\gamma$ represent the drug concentration in the medium (μM) and the sigmoidicity factor, respectively.

#### *Growth rate of cells under the culture conditions*

Cells ($5 \times 10^3$/well) were seeded into 96-well plates as described above, and then cultured in an atmosphere of 95% air and 5% $CO_2$ at 37°C. The proliferation was evaluated at $t$ = 0, 6, 12, 18, 24, 36, 48, 72, 96, 120, 144, and 168 h with a WST-1 assay, and a growth curve for each cell was drawn. The doubling-time of cell growth was calculated from the logarithmic phase of the curve [8].

### Cellular kinetics of platinum

#### *Cellular accumulation*

Cells ($1 \times 10^6$/well) were precultured for 48 h in 6-well plates. They were washed twice with a warmed Hanks' balanced salt solution (HBSS) containing 25 mM HEPES. The experiments were started by addition of fresh HBSS containing 10 μM of cisplatin, carboplatin, or oxaliplatin, and the cells were further incubated for the desired period at 37°C. The reaction was terminated by aspiration of the HBSS from the well, followed by three washes with ice-cold phosphate-buffered saline (PBS). After the accumulation experiments, 1 ml of ice-cold pure water was added, and the cells were frozen over 30 min at −80°C. Cells were defrosted at room temperature, and lysed by an ultrasonic wave [10].

#### *Binding of platinum to DNA*

Cells ($5 \times 10^6$) were precultured for 48 h in 100 mm plastic dish. Cells were washed twice with a warmed HBSS, and the reaction was initiated by addition of HBSS containing 10 μM of cisplatin, carboplatin, or oxaliplatin. After 24 h, the reaction was terminated by removing the HBSS, and then the cells were washed once with ice-cold PBS. Cellular DNA was extracted with a Wizard® SV

genomic DNA purification system (Promega, Madison, WI, USA). Cells were solubilized with 600 μl of lysis buffer, and the lysate was transferred to a Wizard SV minicolumn, and centrifuged at 13,000×*g* for 3 min. The wash solution (650 μl) was added to the same column, and the mixture was centrifuged at 13,000×*g* for 1 min four times. In addition, the column was centrifuged at 13,000×*g* for 2 min to remove ethanol. Next, the column was transferred to a collection tube, 250 μl of nuclease-free water with RNase A solution was added (65°C), and the mixture was incubated for 2 min. DNA was soluted by centrifugation at 13,000×*g* for 1 min. The amount of DNA extracted was evaluated by measuring the absorbance of the DNA solution at 260 nm (A260) with a SmartSpec™ 3000 (Bio-Rad, CA, USA) and calculated using the following equation: A260 × 50 (μg/ml).

### *Quantitative analysis of platinum*

An aliquot (0.8 ml) of cell lysate or DNA solution was vigorously mixed with 40 μl of 69% nitric acid (specific gravity 1.42, Wako) and 200 μl of 20 ng/ml gallium standard solution in 1% nitric acid (Wako), as an internal standard for assaying. The mixture was centrifuged at 10,500 × *g* for 10 min at 25°C, and the supernatant was added to 3 ml of 0.55% nitric acid. The amount of platinum was measured with an inductively coupled plasma mass spectrometer (ICP-MS; ICP-MS8400, Shimadzu, Kyoto, Japan) using a standard solution of platinum for atomic absorption spectrometry (Wako). The recovery rate and coefficient of variation in the reproducibility were approximately 100 and 6.2%, respectively, and the standard curves for the assay showed excellent linearity ($r^2 > 0.999$) from 0.097 to 100 ng/ml. The protein content of the cell lysate was determined by the Lowry method [11] with bovine serum albumin as a standard protein. Protein and DNA contents were used to correct the amount incorporated into cells and DNA-binding of platinum, respectively.

### Real-time RT-PCR

The levels of hCtr1, ATP7A, ATP7B, OCT1, OCT2, and OCT3 mRNA were measured with the real-time reverse transcription (RT)-PCR method using SYBR® green. Cells were cultured for 96 h, and then total RNA was extracted from the cells with a GenElute™ Mammalian Total RNA Miniprep kit (Sigma-Aldrich). Aliquots (1 μg) of total RNA were used for RT, using a PrimeScript™ RT reagent kit (Takara Bio, Shiga, Japan) and a thermal cycler (i-Cycler, Bio-Rad). The RT reaction was conducted in 20 μl of reaction buffer at 37°C for 15 min, and terminated by heating at 85°C for 5 s followed by cooling at 4°C.

Real-time PCR was performed with a 7500 Real-Time PCR system (Applied Biosystems, CA, USA) using SYBR Premix Ex Taq™ (Takara Bio). The primers were designed using the Primer Express® program (Applied Biosystems). Their sequences are shown in Table 1. The reaction was performed at 95°C for 10 s, followed by 40 cycles of 95°C for 5 s and 60°C for 34 s. The dissociation stage was initiated at 95°C for 15 s, followed by 1 cycle of 60°C for 1 min and 95°C for 15 s. The fluorescence of the SYBR green dye was determined as a function of the PCR cycle number, giving the threshold cycle ($C_T$) number at which the amplification reached a significant threshold. The $C_T$ values were used to quantify the PCR product, i.e., the relative expression level of the target gene was expressed as $2^{-\Delta CT}$ [12], and $\Delta C_T$ was calculated by subtracting $C_T$ (control gene: $\beta$-actin) from $C_T$ (target gene).

### Statistical analysis

Comparisons among more than three groups were performed with a non-repeated one-way analysis of variance followed by the Scheffe's *F*-test, and *P*-values of less than 0.05 (two-tailed) were considered significant. The correlation analysis was performed using Pearson's correlation coefficient (*r*).

**Table 1** Sequences of oligonucleotide primers designed for real-time PCR

| | Forward (5′–3′ orientation) | Reverse (5′–3′ orientation) | Accession no. |
|---|---|---|---|
| hCtr1 | ACAAGTCAGCATTCGCTACAATTC | TTGCAGGAGGTGAGGAAAGC | U83460 |
| ATP7A | AGATACTGGGACACTGGAGAAAAA | AGGTCATCCCTTCCACTTTCA | AB117973 |
| ATP7B | TGATTTATAACCTGGTTGGGATACC | ATGAGAGCACCACAGACACAGA | U03464 |
| hOCT1 | TCTTCCATCGTCACTGAGTTCAAC | AGAAGCCCGCATTCAAACAG | BC126364 |
| hOCT2 | TCTACTCTGCCCTGGTTGAATTC | ATGCAGCCCAAGGGTAACG | BC039899 |
| hOCT3 | TAGCCCCATTTCTGCTCTTTC | AGATGGATGCCAGGATACCAA | NM_021977 |
| $\beta$-actin | TCATGAAGTGTGACGTGGACATC | TGCATCCTGTCGGCAATG | NM_001101 |

The primers pairs were designed using Primer Express® software

**Table 2** $IC_{50}$ values for platinum derivatives in seven colorectal cancer cell lines

| Cell line | $IC_{50}$ (μM) | | |
|---|---|---|---|
| | Cisplatin | Carboplatin | Oxaliplatin |
| Caco-2 | 16.7 ± 2.27 | 253 ± 4.72 | 0.671 ± 0.05 |
| DLD-1 | 20.8 ± 0.87 | 212 ± 5.30 | 23.2 ± 3.33 |
| HCT-15 | 29.5 ± 3.89 | 190 ± 11.2 | 2.43 ± 0.15 |
| HCT116 | 6.61 ± 1.04 | 185 ± 10.6 | 0.766 ± 0.03 |
| LS180 | 12.4 ± 2.86 | 170 ± 20.2 | 0.902 ± 0.03 |
| SW620 | 37.2 ± 2.31 | 678 ± 65.6 | 46.4 ± 9.04 |
| WiDr | 13.3 ± 3.89 | 166 ± 19.3 | 2.35 ± 0.05 |

Cells were precultured for 24 h at a density of $5 \times 10^3$ cells/well in 96-well multiplates, and then incubated with platinum derivatives at various concentrations for 72 h. The cytotoxic activity of the derivatives was determined with a WST-1 assay. Each value represents the mean ± SE ($n = 4$)

## Results

### Growth inhibition by platinum derivatives

Table 2 lists the $IC_{50}$ values for cisplatin, carboplatin, and oxaliplatin in seven human colon cancer cells. The $IC_{50}$ ranged from 6.6 to 37 μM for cisplatin, from 166 to 678 μM for carboplatin, and from 0.67 to 46 μM for oxaliplatin. In terms of cytotoxic activity, the rank order was oxaliplatin > cisplatin > carboplatin. On the other hand, the growth curves of cells varied, but the doubling times of cell growth were comparable; Caco-2 (25.9 h), DLD-1 (27.4 h), HCT-15 (18.2 h), HCT116 (23.3 h), LS180 (25.2 h), SW620 (27.0 h), and WiDr (35.5 h).

### Cellular kinetics of platinum

The time course of the cellular accumulation of platinum after exposure to each derivative was examined (Fig. 1). The platinum accumulated in a time-dependent manner, and the amounts differed among the types of cells. In addition, levels were almost the same on treatment with cisplatin and oxaliplatin, but lower in response to carboplatin.

The amount of platinum bound to DNA was examined at 24 h after exposure to the derivatives (Fig. 2). Again, the amounts varied among the cells, being comparable for cisplatin and oxaliplatin, but smaller for carboplatin.

### Expression of copper transporter and organic cation transporter mRNAs

The expression levels of the copper transporters hCtr1, ATP7A, and ATP7B, and the organic cation transporters hOCT1, hOCT2, and hOCT3, were examined (Fig. 3). The copper transporter mRNAs were detected in all the cells used here, though levels differed with the type of cell (Fig. 3a–c). The mRNAs of hOCT1 and hOCT2 expressed in the cells, and these levels also varied (Fig. 3d, e). In the case of hOCT3, mRNA expression was observed in all the cells except HCT116 cells, and the level was remarkably higher in SW620 cells than the others (Fig. 3f).

### Correlation analysis of the factors affecting cytotoxicity

The relationship between the $IC_{50}$ and cellular kinetics of the platinum derivatives was analyzed (Fig. 4). The amount of platinum for 24 h after the treatment with cisplatin decreased followed by an increase in the $IC_{50}$ (Fig. 4a), the two changes showing a weak correlation ($r = -0.599$). In the case of carboplatin, a positive relation was observed (Fig. 4b), but for oxaliplatin, no correlation was observed (Fig. 4c). On the other hand, for cisplatin, a negative correlation between the amount of platinum

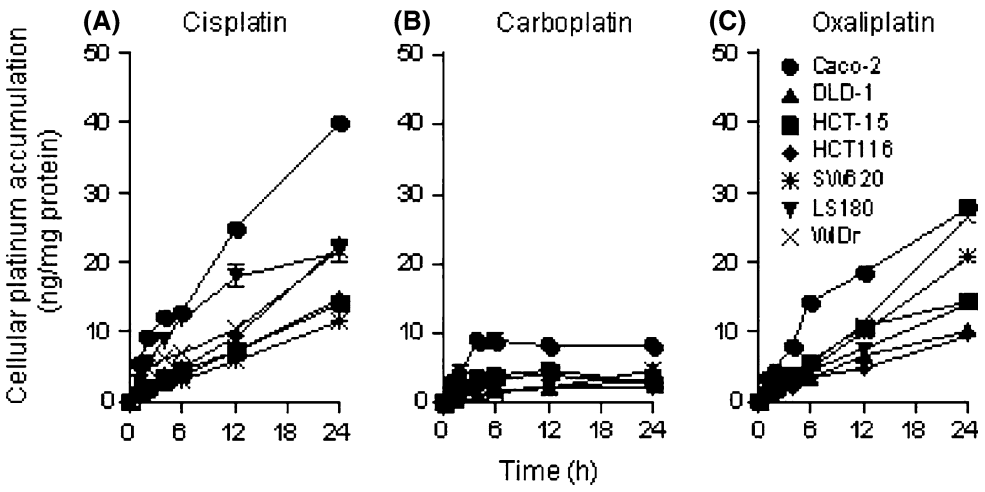


**Fig. 1** Time course of the cellular accumulation of platinum after exposure to platinum derivatives in human colorectal cancer cells. Cells were precultured for 48 h at a density of $1 \times 10^6$ cells/well, and then incubated with 10 μM of cisplatin (**a**), carboplatin (**b**), or oxaliplatin (**c**) for the desired period at 37°C. The cellular amount of platinum was measured using ICP-MS. *Each point* represents the mean ± SE ($n = 3$)

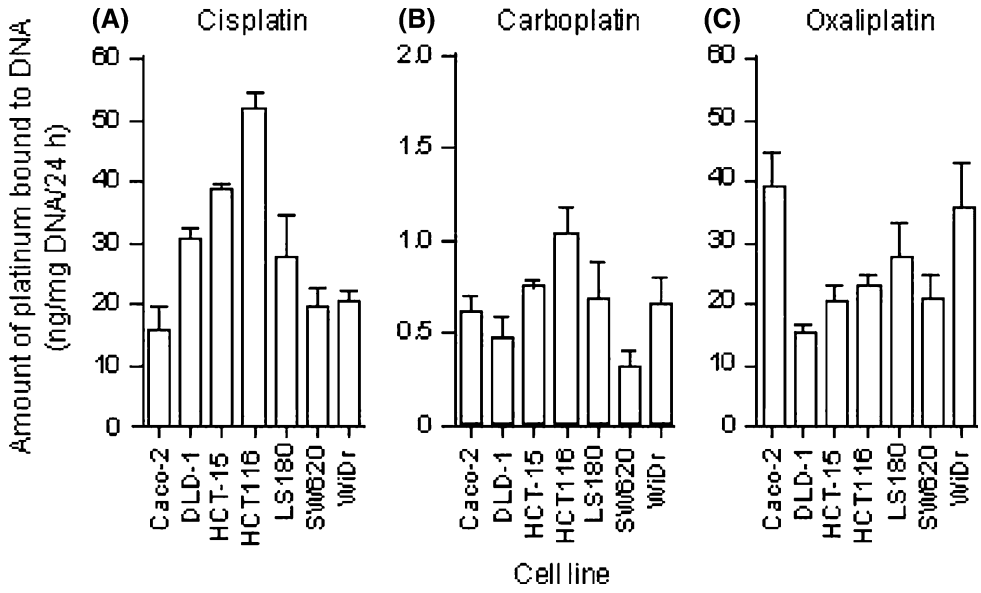


**Fig. 2** Amount of platinum bound to DNA 24 h after exposure to platinum derivatives in human colorectal cancer cells. Cells were precultured for 48 h at a density of $5 \times 10^6$ cells/dish, and then incubated with 10 μM of cisplatin (**a**), carboplatin (**b**), or oxaliplatin (**c**) for 24 h at 37°C. After the incubation, DNA was extracted from the cells and the amount of platinum bound was measured by ICP-MS. *Each bar* represents the mean ± SE ($n = 3$)

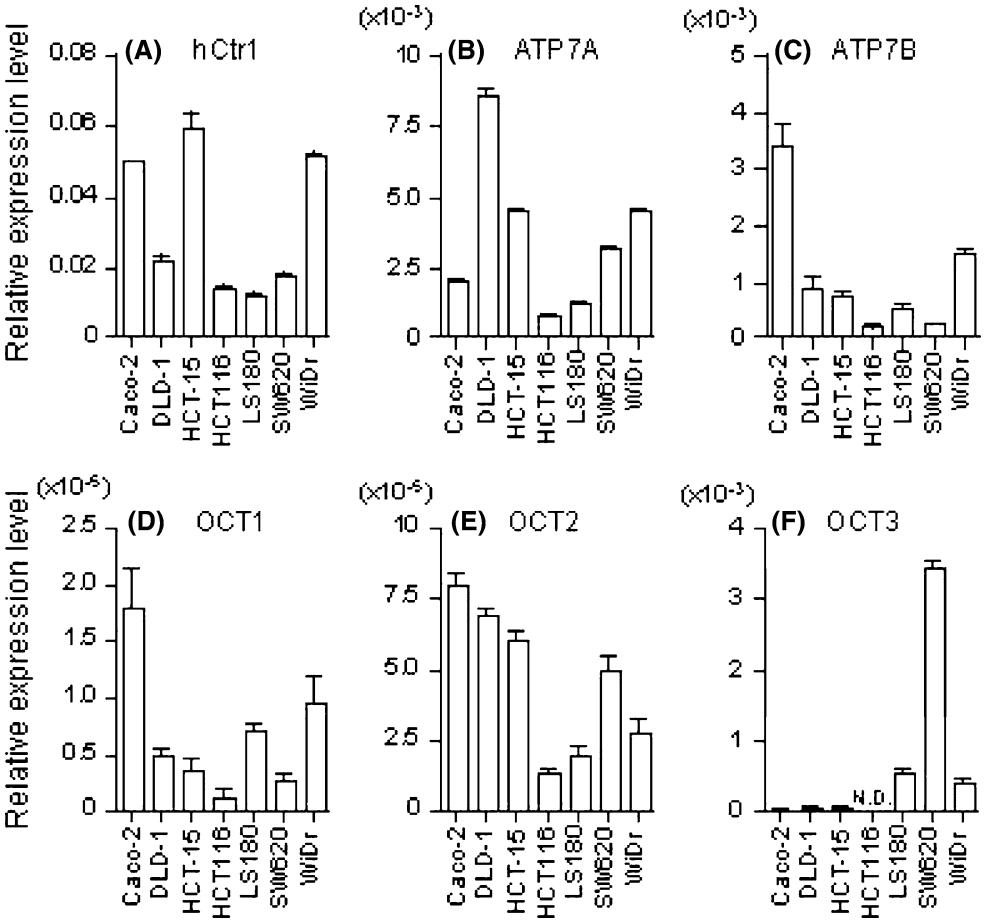


**Fig. 3** Level of copper and organic cation transporter mRNAs in human colorectal cancer cells. Total RNA was extracted from the cells, and the levels of hCtr1 (**a**), ATP7A (**b**), ATP7B (**c**), hOCT1 (**d**), hOCT2 (**e**), hOCT3 (**f**) and *β*-actin mRNAs were measured by a quantitative real-time PCR using SYBR® green. The threshold cycle ($C_T$) values were used to quantify the PCR product, and the relative expression level of the target gene was expressed as $2^{-\Delta CT}$. The $\Delta C_T$ was calculated by subtracting $C_T$ (control gene: *β*-actin) from $C_T$ (target gene). *Each bar* represents the mean ± SE ($n = 3$). *ND* not detected

bound to DNA and $IC_{50}$ was observed, although it was weak (Fig. 4d–f).

Next, the relationship with the levels of transporters and cytotoxicity or cellular kinetics of platinum derivatives was analyzed (Table 3). The $IC_{50}$ values of cisplatin and oxaliplatin showed a positive correlation with the expression level of ATP7A mRNA, but not so the $IC_{50}$ of carboplatin. In the case of hOCT1, a negative correlation with the $IC_{50}$

values of carboplatin and oxaliplatin was found. In addition, a significant correlation was observed between the $IC_{50}$ of cisplatin and level of hOCT2 mRNA, although it was positive.

In the case of cisplatin, the cellular accumulation of platinum showed a higher correlation coefficient for the expression of ATP7B and hOCT1 than for that of the other transporters (Table 3). Oxaliplatin also showed a correlation with the expression levels of ATP7B and hOCT1. In addition, the cellular accumulation of platinum after treatment with carboplatin demonstrated a correlation with the expression levels of ATP7B and hOCT2.

## Discussion

In terms of cytotoxicity, the antitumor platinum derivatives ranked in the order oxaliplatin > cisplatin > carboplatin (Table 2), consistent with a previous report [13]. However, sensitivity varied among the type of cell, although the growth activity in the seven colon cancer cell lines was comparable (a doubling time of 18.2–35.5 h). This discrepancy implies that the sensitivity to platinum derivatives was dependent on factors other than growth activity or rates of DNA synthesis. Therefore, the relationship between the cytotoxicity and cellular kinetics of platinum derivatives was examined in the present study.

The cellular levels of platinum after treatment with cisplatin and oxaliplatin were similar, being higher than with carboplatin (Fig. 1). The hydrophobicity, i.e., ClogP, for cisplatin, carboplatin, and oxaliplatin was −1.68, −0.34, and 0.35, respectively [14], and thus the cellular accumulation of platinum was suggested to be dependent on a transport mechanism, not on passive diffusion. On the other hand, after cisplatin treatment, the amount of platinum accumulated was inversely correlated with the $IC_{50}$ value (Fig. 4). This finding suggested that the cytotoxicity of cisplatin, but not oxaliplatin or carboplatin, was dependent on the cellular accumulation of platinum. To clarify the differences among drugs, the amount of platinum bound to DNA after 24 h of treatment was measured (Fig. 2). In terms of this amount, the derivatives ranked as follows: oxaliplatin ≒ cisplatin > carboplatin, being similar to that in the accumulation experiments. However, in the case of oxaliplatin and carboplatin, a correlation between the $IC_{50}$ and amount of platinum bound to DNA was observed (Fig. 4e, f), and the amount after cisplatin treatment also tended to show a weak correlation with the $IC_{50}$ (Fig. 4d). The cytotoxicity of the platinum derivatives was suggested to be partially dependent on the amount of platinum bound to the DNA. On the other hand, the cellular accumulation and DNA-binding of platinum were less extensive with carboplatin as compared to cisplatin or oxaliplatin (Figs. 1, 2). In addition, the ratio of DNA-binding to the

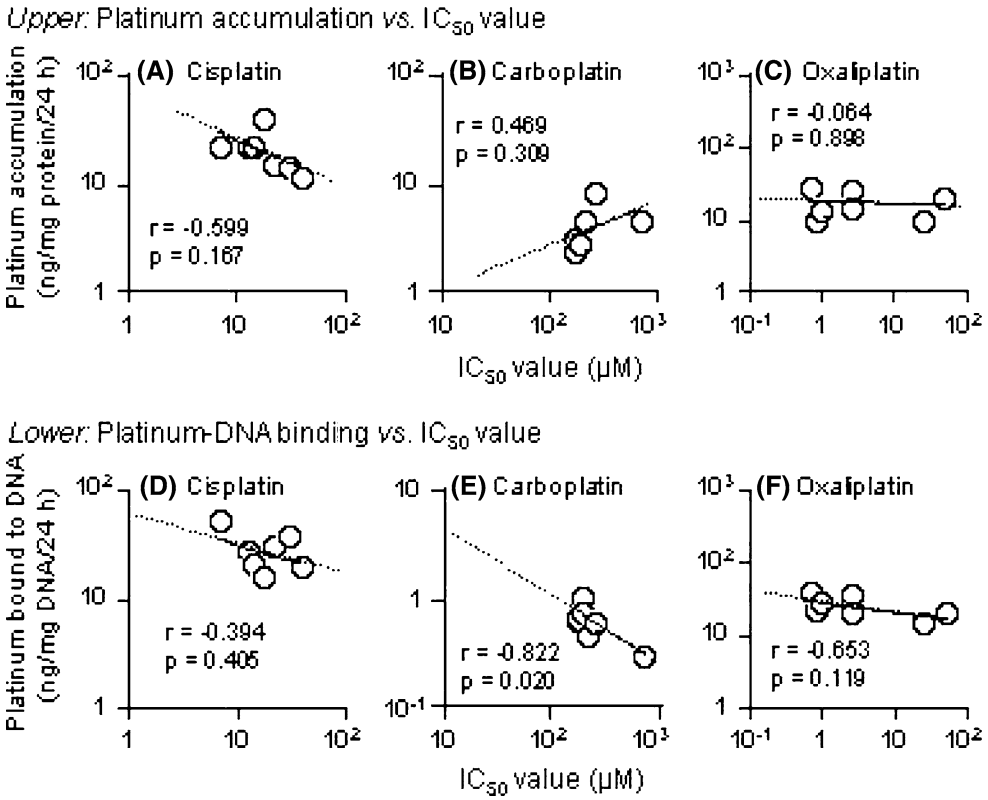


**Fig. 4** Relationship between the $IC_{50}$ and cellular accumulation of platinum derivatives or amount of platinum bound to DNA. The $IC_{50}$ values for platinum derivatives were cited from the data obtained in the WST-1 assay (Table 2). The cellular accumulation of platinum for 24 h and amount of platinum bound to DNA for 24 h were cited from the data in Figs. 1 and 2, respectively. A correlation analysis was carried out using Pearson's correlation coefficient (*r*)

**Table 3** Relationship between levels of transporters and cytotoxicity or cellular kinetics of platinum derivatives

| | $IC_{50}$ value | | | Accumulation | | |
|---|---|---|---|---|---|---|
| | Cisplatin | Carboplatin | Oxaliplatin | Cisplatin | Carboplatin | Oxaliplatin |
| hCtr1 | $r = 0.339$ (0.480) | $r = -0.169$ (0.733) | $r = -0.129$ (0.796) | $r = 0.215$ (0.663) | $r = 0.353$ (0.460) | $r = 0.582$ (0.184) |
| ATP7A | $r = 0.673$ (0.103) | $r = 0.122$ (0.806) | $r = 0.671$ (0.104) | $r = -0.479$ (0.296) | $r = 0.251$ (0.608) | $r = 0.138$ (0.781) |
| ATP7B | $r = 0.061$ (0.904) | $r = -0.322$ (0.491) | $r = -0.309$ (0.522) | $r = 0.625$ (0.143) | $r = 0.529$ (0.239) | $r = 0.590$ (0.175) |
| hOCT1 | $r = 0.125$ (0.802) | $r = -0.186$ (0.707) | $r = -0.246$ (0.615) | $r = 0.572$ (0.194) | $r = 0.438$ (0.347) | $r = 0.702$ (0.081) |
| hOCT2 | $r = 0.773$ (0.040) | $r = 0.373$ (0.434) | $r = 0.438$ (0.348) | $r = -0.101$ (0.839) | $r = 0.719$ (0.070) | $r = 0.351$ (0.463) |
| hOCT3 | $r = 0.134$ (0.816) | $r = 0.544$ (0.291) | $r = 0.376$ (0.493) | $r = -0.398$ (0.465) | $r = -0.305$ (0.585) | $r = 0.239$ (0.673) |

The *r* represents Pearson's correlation coefficient, and the figure in parentheses is the *P*-value

cellular accumulation of platinum was markedly lower in carboplatin than the other derivatives (data not shown). These findings demonstrated that the cellular kinetics of carboplatin were dissimilar to those of cisplatin and oxaliplatin. However, the detailed mechanism remains unclear, and thus the further investigation is needed.

Some copper transporters such as the uptake transporter hCtr1, and efflux transporters ATP7A and ATP7B, have been demonstrated to control the cellular kinetics of platinum derivatives, suggesting a contribution to the sensitivity of cells [15–17]. In the present study, the expression of hCtr1, ATP7A, and ATP7B mRNA was observed in all the cells, but their levels differed among the cell types (Fig. 3). Therefore, the relationship between these levels and the cellular kinetics or cytotoxicity of platinum derivatives was examined (Table 3). The expression of hCtr1 mRNA showed a positive correlation with the accumulation of platinum, but did not correlate with the $IC_{50}$. These results imply that the level of hCtr1 mRNA alone could not be used to predict sensitivity to platinum derivatives. Some reports also clarified that the incorporation of cisplatin and oxaliplatin into cells was dependent on a mechanism not involving hCtr1 [3, 18, 19].

ATP7A and 7B were also reported to control the intracellular kinetics of platinum, causing less accumulation [15]. The expression of ATP7A mRNA was positively correlated with the $IC_{50}$ values of cisplatin and oxaliplatin (Table 3), and especially with cisplatin, the cellular amount of platinum showed a correlation with the expression of ATP7A mRNA (Table 3). It may be that the cytotoxicity of cisplatin was reduced *via* the ATP7A-mediated efflux of platinum. In the clinical setting, the expression of ATP7A was reported to induce a reduction in the survival rates of patients receiving cisplatin-containing regimens [20], supporting the present findings. However, the expression of ATP7A mRNA might not be a predictive factor for sensitivity to carboplatin and oxaliplatin. On the other hand, the expression of ATP7B mRNA was correlated with the cellular amount of platinum, but not the $IC_{50}$ (Table 3). Samimi et al. reported that ATP7B acted to accumulate cisplatin and carboplatin, resulting in their isolation from DNA, but not oxaliplatin [21]. Overall, the expression of ATP7B mRNA was suggested not to be enough to predict sensitivity to platinum derivatives.

Recently, organic cation transporters, hOCT1, hOCT2, and hOCT3, as well as copper transporters, have been reported to affect the cellular kinetics of platinum derivatives [3, 22]. The expression of hOCT1 mRNA showed a positive correlation with the cellular amount of platinum (Table 3). Notably, the expression showed a correlation with the $IC_{50}$ for oxaliplatin, different from cisplatin and carboplatin. This raises the possibility that oxaliplatin was incorporated into the cell *via* hOCT1, an interpretation supported by other reports [3, 22].

In conclusion, the cytotoxicity of platinum derivatives varied with the kind of drug as well as the type of cell. The sensitivity to platinum derivatives of tumor cells was suggested to be controlled in part by the amount of platinum bound to DNA. In addition, the cellular accumulation of platinum and the level of ATP7A mRNA might be factors affecting sensitivity to cisplatin, while the expression levels of ATP7A and hOCT1 mRNAs might affect sensitivity to oxaliplatin. The present findings will provide useful information for the individualization and optimization of dosages in cancer chemotherapy with platinum derivatives.

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