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Cellular pharmacology of cisplatin in relation to the expression of human copper transporter CTR1 in different pairs of cisplatin-sensitive and -resistant cells

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Abstract

The molecular mechanism of cisplatin uptake remains poorly defined and impaired drug accumulation may be implicated in the acquisition of resistance to cisplatin. Thus, we used cell lines of different tumor types (ovarian carcinoma A2780 and IGROV-1, osteosarcoma U2-OS, cervix squamous cell carcinoma A431) and stable cisplatin-resistant sublines, exhibiting variable levels of resistance (between 2.5 and 18.4), to investigate the mechanisms of cellular accumulation of cisplatin. Among the resistant lines we found that reduced cisplatin uptake was a common feature and ranged between 23 and 76%. In an attempt to examine the role of human copper transporter 1 (CTR1) in cisplatin accumulation by human cells, we selected the well characterized A431 cell line and the resistant variant A431/Pt. As compared with A431/Pt cells, A431/Pt transfectants overexpressing CTR1 (3.4-fold) exhibited increased uptake of copper, thereby supporting the expression of a functional transporter. However, no changes in cisplatin uptake and cellular sensitivity to drug were observed. Also overexpression of CTR1 in A431 cells did not produce modulation of cisplatin accumulation. An analysis of the expression of other factors that could affect drug accumulation indicated that A431/Pt cells displayed increased expression of ATPase, Cu^{2+} transporting, alfa polypeptide. In conclusion, our results indicate that the overexpression of a functional CTR1 in a human cell line characterized by impaired cisplatin uptake fails (a) to restore cellular drug accumulation to the level of the parental cell line and (b) to modulate cisplatin sensitivity. Our data are consistent with the interpretation that the defects in cellular accumulation by resistant cells are not mediated by expression of CTR1, that plays a marginal role, if any, in cisplatin transport.

Keywords: Copper transporter 1; Copper transporter 2; ATPase; Cu^{2+} transporting polypeptide; Cisplatin; Cellular resistance

1. Introduction

Cisplatin (DDP) is a chemotherapeutic agent used for the treatment of a variety of solid tumors including squamous cell carcinomas [1]. The development of resistance to DDP during treatment is common and constitutes a major obstacle to the cure of sensitive tumors. Resistance to DDP is thought to be due to the selection of drug-resistant cells that arise through spontaneous somatic mutation [2]. Determination of in vitro sensitivity to DDP of tumor cell lines or samples from patients before and after treatment with DDP indicated that only modest level of resistance may be sufficient for treatment failure [3–5]. This is consistent with the rapid emergence of low levels of DDP resistance documented in human tumor xenografts [6].

To understand the clinically relevant mechanisms of resistance, preclinical studies have been aimed at clarifying the biochemical/molecular alterations of resistant cells. These studies did not conclusively identify the bases of cellular resistance to DDP, but they contributed to define the multifactorial alterations involved, such as reduced drug accumulation [7,8], increased detoxification through thiol-mediated mechanisms [9,10], reduced DNA platination and enhanced DNA–platinum adduct removal [11].

Abbreviations: DDP, cisplatin; CTR1, copper transporter 1; CTR2, copper transporter 2; ATP7A, ATPase, Cu^{2+} transporting, alfa polypeptide; ATP7B, ATPase, Cu^{2+} transporting, beta polypeptide; PBS, phosphate-buffered saline; RI, resistance index

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Moreover, a frequently identified alteration of cells with acquired DDP resistance both in vitro and in vivo involves impaired drug uptake [5,7,12–18].

The mechanism of cellular DDP accumulation is poorly defined. Several evidences suggest that drug uptake is mediated by a transport mechanism or channel [19–22]. The copper transporter 1 (CTR1) has been recently implicated in mediating the DDP uptake and acquisition of DDP resistance in yeast, mouse, and mammals [23,24]. Conflicting results were obtained by Chauhan et al. [25] in the human epidermoid carcinoma cell line KB-3-1 and in the DDP-resistant variant KB-CP20, as the overexpression of human CTR1 gene in KB-CP20 failed to change DDP accumulation.

In an attempt to examine the role of human CTR1 as a determinant of DDP resistance/sensitivity and accumulation in human cells, we used the human cervix squamous cell carcinoma cell line A431 and the DDP-resistant variant A431/Pt. In particular, we cloned and overexpressed the human CTR1 cDNA in A431 and A431/Pt cells. We found that the overexpression of CTR1 failed to increase DDP accumulation and to change DDP sensitivity in both cell lines. Our results support that the defects in cellular accumulation by resistant cells are not mediated by expression of CTR1, that plays a marginal role, if any, in cisplatin transport.

2. Materials and methods

2.1. Cell lines and transfection

The human ovarian carcinoma A2780 and IGROV-1, osteosarcoma U2-OS, cervix squamous cell carcinoma A431 cell lines and their DDP-resistant sublines (A2780/CP, IGROV-1/Pt1 and IGROV-1/CP, U2-OS/Pt, and A431/Pt obtained by in vitro selection with DDP) were maintained at 37 °C in RPMI-1640 supplemented with 10% fetal calf serum.

A431 and A431/Pt cells were transfected using the Gene PORTER reagent (Gene Therapy Systems, San Diego, CA, USA) according to the manufacturer's protocol. Cells were seeded in 6-well plates and 24 h after seeding DNA plasmids and Gene PORTER transfection reagent were added. Stable transfectants were selected using G418 (400 μg/ml; Calbiochem, San Diego, CA, USA) and G418-resistant cells were picked for further analysis.

2.2. Construction of mammalian pDEST12.2CTR1 plasmid

The pDEST12.2CTR1 vector was prepared using the Gateway cloning technology (Invitrogen, Paisley, UK). Briefly, the PCR product of full length human CTR1 cDNA was obtained using the RNA of A431 cells as a template and primers flanked by *attB* recombination sites. Purified cDNA carrying *attB* sites was introduced into the pDONR201 plasmid via recombination activity of *Escherichia coli* bacteriophage lambda recombination protein (CLONASE Enzyme Mix), thereby generating the pDONR201CTR1 plasmid. The vector was employed to introduce CTR1 cDNA into the mammalian destination vector pDEST12.2 using the clonase enzyme. The obtained expression vector was designated pDEST12.2CTR1 and was checked by sequencing the full length insert.

2.3. RNA extraction, reverse transcriptase-polymerase chain reaction (RT-PCR) and DNA sequencing

Total RNA was isolated with the TRIzol reagent (Invitrogen) according to the manufacturer's protocol. One microgram of total RNA was converted to cDNA with the Superscript First-Strand Synthesis System (Invitrogen). The cDNAs were used to amplify the CTR1, CTR2 (copper transporter 2), ATP7A (ATPase, Cu^{2+} transporting alpha polypeptide), and ATP7B (ATPase, Cu^{2+} transporting, beta polypeptide) genes (CTR1 primers: 5′-agctatatggactccaacag-3′ and 5′-cgttgtaggtcatgaagatg-3′; CTR2 primers: 5′-cctgcgcagtcaccatggcg-3′ and 5′-tgttgtaggacattacggcc-3′; ATP7A primers: 5′-gcctgcgtacgtggatttat-3′ and 5′-tcaatggtccaaacacagga-3′; ATP7B primers: 5′-actggtggaagaggctcaga-3′ and 5′-ccggatgatcacctctgtct-3′). In each sample forward and reverse primers for β-actin (5′-gaaactaccttcaactccatc-3′ and 5′-ggcggctccatcctggcctcg-3′) were added and the amplified product was used as a control for the densitometric analysis. PCR conditions were as follows: initial denaturation at 95 °C for 3 min, followed by 30 cycles at 95 °C for 1 min, 62 °C (for CTR1)/58.5 °C (for CTR2)/45 °C (for ATP7A and ATP7B) for 1 min, 72 °C for 1 min, and finally 72 °C for 10 min. The cDNAs from A431 and A431/Pt cells were also used to sequence CTR1 full length cDNA using appropriate primers (5′-atggatcattcccaccatatgg-3′ and 5′-tcaatggcaatgctctgtgatatc-3′).

2.4. Northern blot analysis

RNA extraction was performed using TRIzol (Invitrogen). Fifteen micrograms of RNA from each sample were fractionated onto 1% agarose gel and transferred overnight on nitrocellulose membranes. Filters were hybridized with a cDNA probe corresponding to the complete CTR1 ORF labeled with [α-^{32}P]dCTP by random hexamer priming (Amersham Biosciences, Little Chalfont, UK). The filters were washed (0.5× SSC, 0.1% SDS, 65 °C, 10 min) and exposed to autoradiography films with intensifying screens at −80 °C. The membranes were then rehybridized with a human β-actin probe labeled with [α-^{32}P]dCTP as described above. The films were scanned, and densitometric analysis was performed with the PhosphoImager dedicated software (ImageQuant, Molecular Dynamics, Sunnyvale, CA, USA).

2.5. DDP and copper cellular uptake

Exponentially growing cells were seeded in 10 cm diameter dishes in triplicate and, 48 h later, they were exposed to DDP or copper for 1 h. Cell monolayers were then washed with ice-cold phosphate-buffered saline (PBS), scraped, harvested and dissolved in 1 N NaOH (for DDP detection) or in 0.015 mg Pd and 0.01 mg $Mg(NO_3)_2$ (for copper). Total cellular platinum or copper content was determined by flameless atomic absorption spectroscopy (Model 3300, Perkin Elmer for DDP and Zeeman for copper). Cellular platinum or copper levels were expressed as ng/10^6 cells, with cell number determined by counting parallel cultures.

2.6. Cellular sensitivity to DDP or $CuSO_4$

Cell sensitivity was assessed by growth-inhibition assays [26]. Briefly, cells in the logarithmic phase of growth were harvested and seeded into 6-well plates. Twenty-four hours after seeding, cells were exposed to DDP (for 1 h) or $CuSO_4$ (for 1 h or 24 h) and harvested 72 h later. Cells were than counted with a Coulter counter. IC_{50} is defined as the concentration causing a 50% inhibition of cell growth as compared with control.

3. Results

3.1. DDP and copper sensitivity and DDP uptake in human cell lines of different tumor types

Cellular sensitivity to DDP (1 h exposure) and copper ($CuSO_4$, 1 and 24 h exposure) was assessed by growth-inhibition assay in human cell lines of different tumor types and in the corresponding DDP-resistant sublines. The relative IC_{50} are reported in Table 1. A2780/CP, U2-OS/Pt, IGROV-1/Pt, IGROV-1/CP, and A431/Pt sublines were, respectively, 18.4, 4.7, 11.5, 9.9, and 2.5-fold resistant to DDP as compared with the parental cell lines.

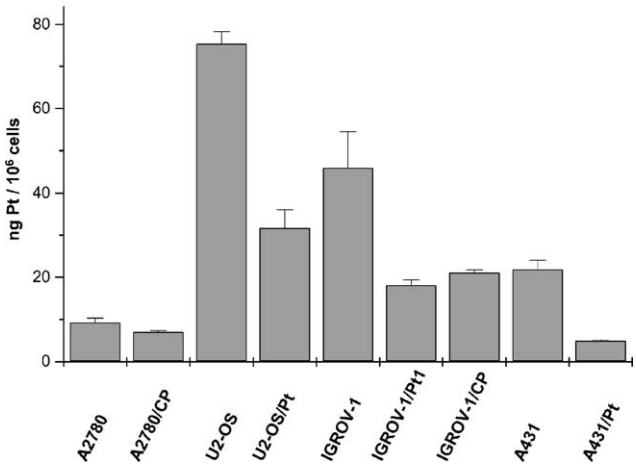

Fig. 1. Cellular Pt accumulation after 1 h exposure to 100 μM DDP. Each data point represents the mean (±S.D.) of three independent experiments.

No change in copper sensitivity was observed for DDP-sensitive cells when compared with the corresponding DDP-resistant cell lines at 1 h exposure. However, cross resistance to copper (two-fold) was observed in A431/Pt cells at 24 h exposure.

Cellular accumulation of DDP after 1 h exposure to a concentration of 100 μM is shown in Fig. 1. In all the resistant variants, a substantial impairment of platinum accumulation was found. DDP uptake in the resistant sublines was reduced by 24% in A2780/CP, 58% in U2-OS/Pt, 61% in IGROV-1/Pt1, 54% in IGROV-1/CP, and 77% in A431/Pt cells as compared to parental cell lines. There was no association between IC_{50} values and cellular platinum accumulation for either DDP-sensitive or -resistant cells.

3.2. Overexpression of CTR1

Northern blot analysis was used to determine the relative CTR1 mRNA levels in the selected cell line systems. Overall, the level of expression in the different cell lines normalized with respect to actin was similar (Fig. 2A

Table 1
Sensitivity of different human cancer cell lines to DDP or $CuSO_4$[a]

Cell line	DDP IC_{50} (μM)		$CuSO_4$ IC_{50} (mM)		
	1 h	RI	1 h	24 h	RI
A2780	4.7 ± 1.33		2.08 ± 0.59	0.26	
A2780/CP	86.66 ± 43.3	18.4	1.99 ± 0.35	0.29	1.1
U2-OS	18.7 ± 5.33		2.9 ± 0.28	0.64 ± 0.07	
U2-OS/Pt	88.7 ± 16.33	4.7	3.25 ± 0.64	0.56 ± 0.23	0.88
IGROV-1	9.64 ± 5.62		2.6 ± 0.28	0.54 ± 0.15	
IGROV-1/Pt1	111.11 ± 19.24	11.5	2.55 ± 0.22	0.49 ± 0.042	0.9
IGROV-1/CP	96.11 ± 29.36	9.9	3.1 ± 0.42	0.44 ± 0.007	0.81
A431	35.7 ± 8.3		2.75 ± 0.21	0.17 ± 0.007	
A431/Pt	90 ± 18.33	2.5	2.7 ± 0.14	0.34 ± 0.021	2

[a] Cell sensitivity was assessed by growth-inhibition assay. Cells were exposed to DDP (1 h) or $CuSO_4$ (1 and 24 h) and counted 72 h later. RI, resistance index.

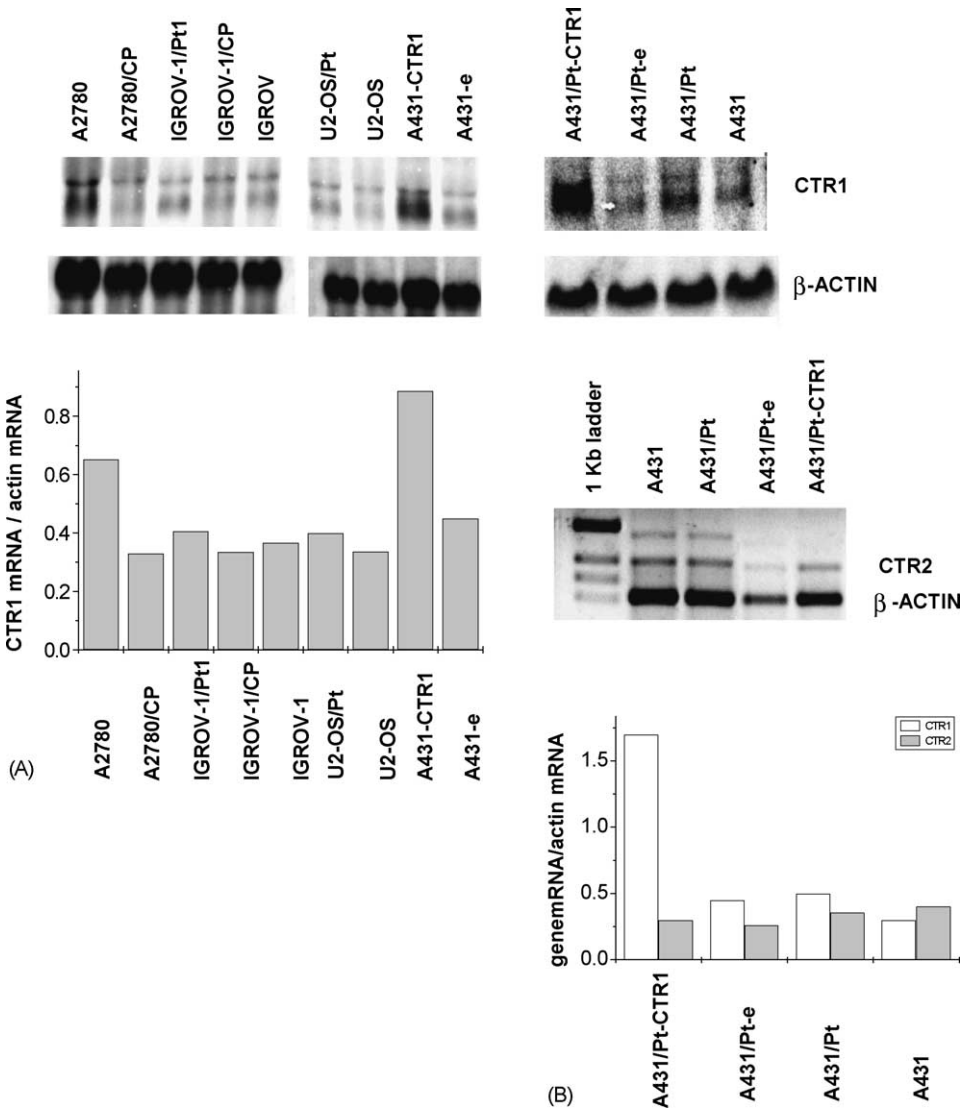

Fig. 2. (A) Northern blot analysis of CTR1. Total RNA was used, and filters were hybridized with a DNA probe corresponding to the complete CTR1 ORF. Control loading is shown by β-actin. The relative expression levels are reported in the corresponding histogram. (B) RT-PCR of CTR2 and expression levels of CTR1 and CTR2 gene. The mRNA levels were analyzed by RT-PCR and were normalized with respect to β-actin.

and B). Since among the tested cell lines, the A431/Pt cells exhibited the highest level of DDP accumulation impairment (77% decrease), we selected this cell system for further studies.

In an attempt to examine the role of human CTR1 gene as a determinant of DDP accumulation and sensitivity/resistance in human cells, we cloned and overexpressed the copper transporter in A431 and A431/Pt cell lines. Since the CTR1 gene from A431 and A431/Pt was sequenced and no mutations were found, the cDNA of the full length CTR1 obtained from A431 was cloned in the mammalian pDEST12.2 expression vector, which was then transfected in A431 and A431/Pt cells. Polyclonal populations of empty vector (A431-e, A431/Pt-e) and CTR1-transfected cells (A431-CTR1, A431/Pt-CTR1) were selected for further analysis.

RT-PCR and Northern blot analysis were used to determine the relative mRNA levels. As reported in Fig. 2A and B, A431/Pt cells exhibited a 1.7-fold increased expression of CTR1 when compared with the parental A431 cell line, while A431/Pt-CTR1 cells displayed a CTR1 expression 3.4-, 5.7-, and 3.8-fold higher than A431/Pt, A431, and A431/Pt-e cells, respectively. RT-PCR analysis reveled that A431-CTR1 cells exhibited increased expression of CTR1 when compared with the A431-e cells (not shown).

We also tested the functionality of CTR1 in A431/Pt-CTR1 cells by examining copper accumulation. A431/Pt-e and A431/Pt-CTR1 cells were exposed for 1 h to 10 and 30 μM $CuSO_4$. As shown in Fig. 3, A431/Pt-CTR1 cells exhibited a 2.1-fold increase in copper basal level as compared with A431/Pt-e cells ($P < 0.05$, ANOVA). When treated with 10–30 μM $CuSO_4$, A431/Pt-CTR1 cells exhibited 1.8/3.3-fold increase in copper accumulation as compared with A431/Pt-e cells ($P < 0.05$, ANOVA).

Copper uptake in mammalian cells is thought to be principally mediated by CTR1, but as human CTR2 shares

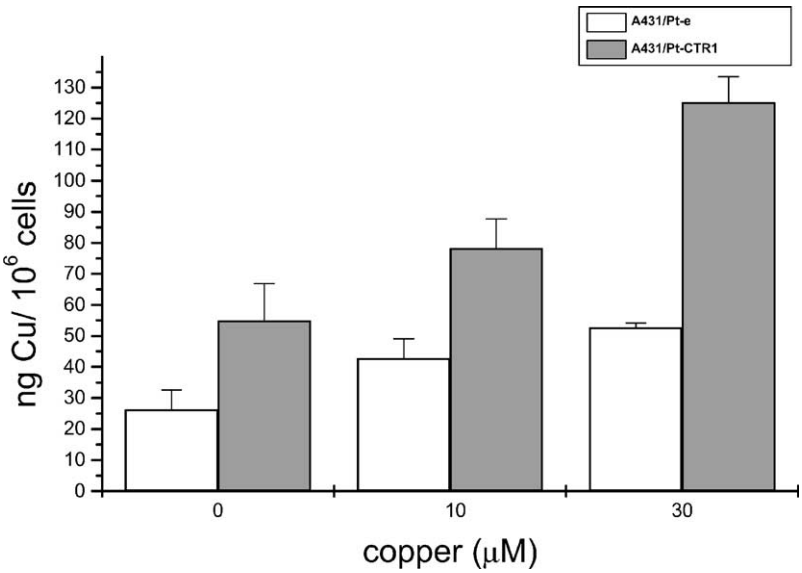

Fig. 3. Cellular copper accumulation after 1 h exposure to 0–30 μM $CuSO_4$. Each data point represents the mean (±S.D.) of three independent experiments.

a high degree of homology with CTR1, we also measured CTR2 gene expression. The expression levels of CTR2 were not altered in all the cell lines, as shown by RT-PCR analysis (Fig. 2B). An analysis of copper sensitivity in CTR1-overexpressing A431/Pt-CTR1 cells indicated no change after 1 h exposure to $CuSO_4$ (Table 2).

3.3. Expression of ATP7A and ATP7B

The relative mRNA level of the copper omeostasis proteins ATP7A and ATP7B was examined by RT-PCR analysis (Fig. 4). When compared to the parental cell lines, A431/Pt cells showed a increased espression of ATP7A, whereas the expression level was reduced in IGROV-1/CP and U2-OS/Pt cells. No change in mRNA level was found in the A2780-A2780/CP cells. The relative mRNA level of ATP7B was decreased only in the IGROV-1/Pt1.

3.4. DDP sensitivity and uptake in cells overexpressing CTR1

To address whether DDP sensitivity/resistance is dependent on CTR1 expression levels, we examined sensitivity of A431-CTR1 and A431/Pt-CTR1 cells to DDP. The analysis was performed also on the transfected parental cell line as it is possible that the CTR1 gene may express well but may not function normally in A431/Pt cells [27]. As shown in Table 2, no change in drug antiproliferative effect was observed in A431-e–A431/Pt-e and A431-CTR1–A431/Pt-CTR1 cells as compared with A431 and A431/Pt cells after 1 h DDP exposure. The measurement of DDP accumulation after 1 h exposure to drug concentrations ranging from 10 to 500 μM revealed an approximately two-fold lower accumulation in DDP-resistant than in sensitive cells, but, under any tested condition (i.e exposure to low and high concentrations), no increase in platinum uptake was observed for cells overexpressing CTR1 when compared with cells transfected with empty vector ($P > 0.05$, ANOVA) (Fig. 5A and B, Table 3). In particular, statistical analysis revealed a significant reduced DDP uptake when comparing A431 with A431/Pt cells ($P < 0.05$, ANOVA), whereas no change in platinum uptake was observed for cells overexpressing CTR1 when compared with cells transfected with empty vector or with A431/Pt cells ($P > 0.05$, ANOVA).

Table 2
Sensitivity of A431, A431/Pt, and CTR1 overexpressing A431, A431/Pt cells to DDP and $CuSO_4$[a]

Cell line	DDP IC_{50} (μM) (mean ± S.D.)	$CuSO_4$ IC_{50} (mM) (mean ± S.D.)
A431	35.7 ± 8.3	2.75 ± 0.21
A431/Pt	90 ± 18.33	2.7 ± 0.14
A431/Pt-e	98.5 ± 12	2.03 ± 0.47
A431/Pt-CTR1	95 ± 21	2.04 ± 0.367
A431-e	35.2 ± 14.34	1.85 ± 0.22
A431-CTR1	24.17 ± 9.81	1.96 ± 0.29

[a] A431 and A431/Pt cells were transfected with CTR1 containing plasmid or empty vector and sensitivity of the obtained stable transfectants (A431-CTR1, A431-e, and A431/Pt-CTR1, A431/Pt-e) and of A431 and A431/Pt cells was assessed by growth-inhibition assay. Cells were exposed for 1 h to DDP or $CuSO_4$ and counted 72 h later.

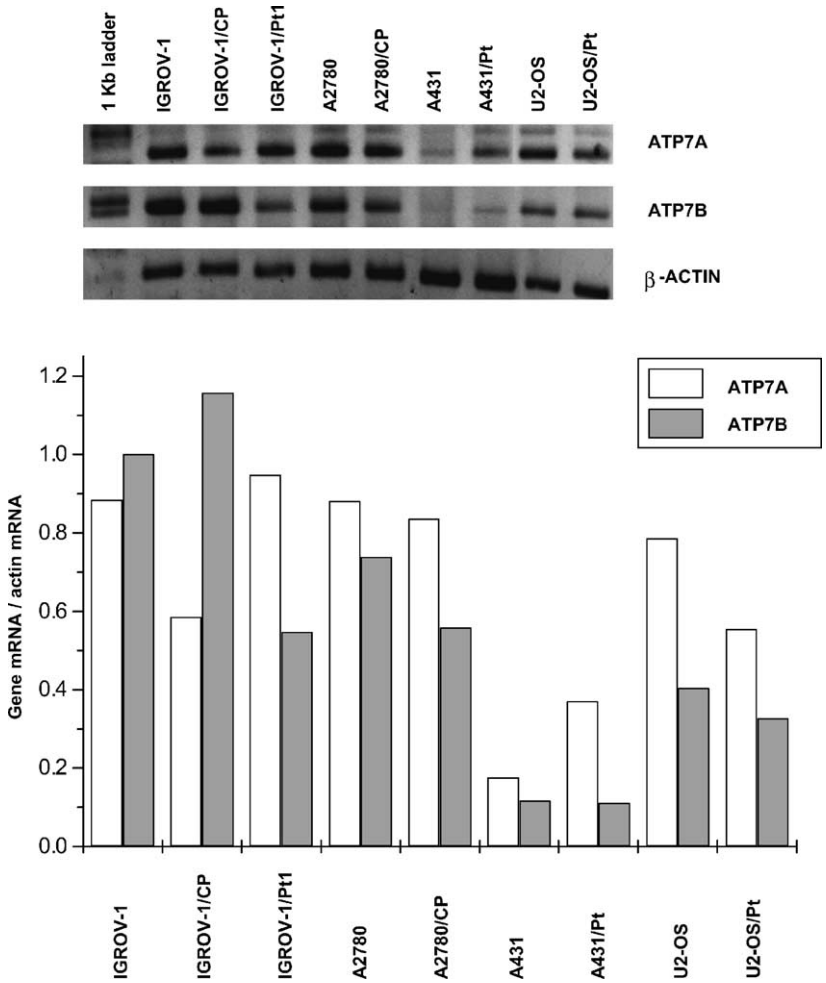

Fig. 4. Expression level of ATP7A and ATP7B gene. The mRNA levels were analyzed by RT-PCR and were normalized with respect to β-actin.

4. Discussion

Many studies were carried out for clarifying the mechanisms of DDP resistance, but the molecular mechanisms that underlie DDP resistance are poorly understood. It is evident that different features including pre-target events and cell response to cisplatin can contribute to the resistant phenotype [28]. In particular, reduced susceptibility to apoptosis has been associated with drug resistance in the model systems used in the present study [29–31]. Regarding pre-target events, reduced drug accumulation is a common alteration found in DDP-resistant cell lines. In this context, a large effort has been made to define how DDP enters cells [7], since reduced drug accumulation is a common alteration found in DDP-resistant cell lines [21,31–35].

Therefore, in the present study, we examined the cellular pharmacology of cisplatin in pairs of human cell lines of different tumor types, including DDP-resistant variants, in relation to uptake impairment. In the tested cell systems, reduced DDP uptake was a common feature accompanying the acquisition of resistance. The greatest magnitude of DDP uptake impairment and the cross-resistance to copper, though weak, observed in the DDP-resistant cervix squamous cell carcinoma A431/Pt cells led us to select this system for further studies aimed at clarifying the role of CTR1 in DDP uptake.

Table 3
Pt accumulation of A431, A431/Pt and CTR1 overexpressing A431/Pt cells after exposure to low cisplatin concentrations[a]

DDP (μM)	A431	A431/Pt	A431/Pt-e	A431/Pt-CTR1
10	0.76 ± 0.002	0.59 ± 0.014	0.87 ± 0.06	0.83 ± 0.3
25	1.91 ± 0.02	1.42 ± 0.047	2.05 ± 0.4	1.78 ± 0.4

[a] Pt accumulation was measured by atomic absorption after 1 h exposure to cisplatin of A431/Pt cells transfected with CTR1/empty vector (A431/Pt-CTR1, A431/Pt-e) and of A431 and A431/Pt cells. A431/Pt vs. A431 Pt level at 10 and 25 μM; $P < 0.05$(ANOVA).

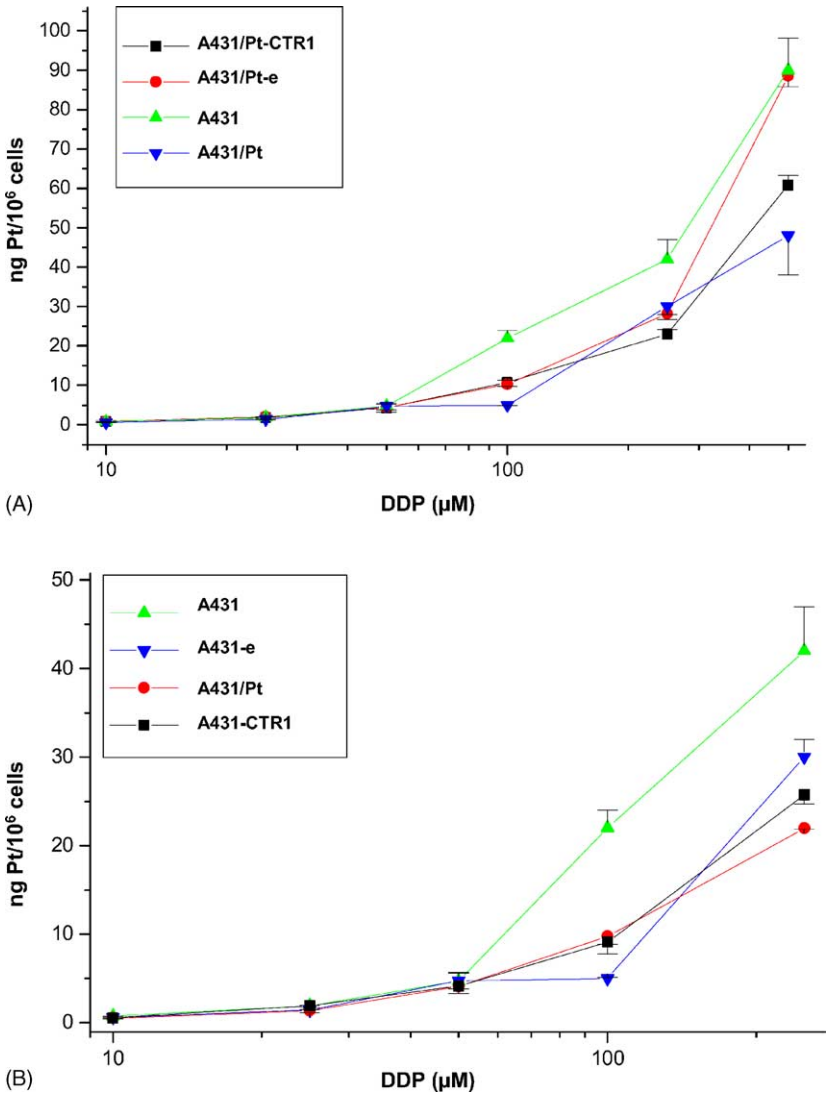

Fig. 5. Cellular Pt accumulation after 1 h exposure as a function of DDP concentration. (A) DDP uptake in A431, A431/Pt, A431/Pt-CTR1, and A431/Pt-e. Statistical analysis revealed no significant difference between A431-e and CTR1 overexpressing A431/Pt cells. (B) DDP uptake in A431, A431/Pt, A431-e, and A431-CTR1. Statistical analysis revealed no significant difference between A431-e and CTR1 overexpressing A431 cells. Each point represents the mean (±S.D.) of three independent experiments.

The CTR1 has been implicated in mediating the uptake and acquisition of resistance to DDP in yeast, mouse and mammals [23,24,36] but conflicting data recently emerged in contrast with such a hypothesis [25]. In fact, results obtained by Chauhan et al. in the human epidermoid carcinoma cell line KB-3-1 and in the DDP-resistant variant KB-CP20 revealed no change in DDP accumulation when CTR1 was overexpressed in KB-CP20 cells.

In the present study, no mutations were detected in any exon of CTR1 in A431 and A431/Pt cells. Thus, we cloned and overexpressed the human transporter in the A431/Pt subline. RT-PCR analysis demonstrated the overexpression of CTR1 mRNA in the A431/Pt-CTR1 cells. Indeed, in overespressing cells, increased CTR1 level stimulated copper uptake in a concentration-dependent manner, thereby confirming overexpression of a functional protein.

Although the introduction of exogenous CTR1 leads to an increase in copper uptake in A431/Pt cells no changes in platinum accumulation were observed. This is consistent with the finding that, in comparison with A431/Pt, no alteration of sensitivity to DDP was observed after 1 h drug exposure for cells overexpressing CTR1 and cells transfected with empty vector. These results indicate lack of a significant involvement of the CTR1 in the DDP trafficking of resistant cells. It could be speculated a greater membrane permeability for sensitive cells in comparison with the DDP-resistant variant, and this feature could be prevalent for higher DDP concentration. To exclude that lack of increase in platinum accumulation after CTR1 transfection could be due to possible alterations of cisplatin-resistant cells, we also transfected the parental cell line, but again, no change in platinum accumulation/DDP sensitivity was found.

An analysis of the involvement of other factors (ATP7A, ATP7B, CTR2), in addition to CTR1, in regulating cisplatin levels indicated up-regulation of ATP7A in A431/Pt

cells. This feature is consistent with the observed cross resistance between cisplatin and copper and with the reduced copper accumulation in A431/Pt cells as compared to A431 cells (not shown), because ATP7A functions in a pathway that leads to copper export. In this regard, increased expression of copper transporting P-type adenosine triphosphatases have been associated with cellular resistance to DDP [37] and have been proposed as chemoresistance markers in ovarian carcinoma [38]. Interestingly, ATP7A up-regulation has been related to poor survival in ovarian cancer patients [39]. Thus, although a role for CTR1 per se in A431/Pt cells can be excluded, the results of our study suggest that cellular pharmacology of DDP can be altered by other components of copper homeostasis, specifically ATP7A. However, increased expression of copper efflux pumps does not appear a common feature of the different DDP-resistant cell systems.

Several transporters (e.g., arsenical transporters) or channels (gated and water channels) as well as other mechanisms (e.g., passive permeability, pinocytosis, or receptor-mediated endocytosis) has been proposed as being implicated in regulating DDP accumulation [7,34,40–42], but conclusive evidence is lacking. We recently reported a lack of involvement of the human aquaporin 9 in the DDP uptake in ovarian carcinoma cells, and suggested that DDP accumulation occurred via passive diffusion [43]. The available evidence indicates that both passive diffusion and carrier-mediated transport can act as mechanisms of DDP influx [43–45]. DDP accumulation can be modulated by several agents (e.g., protein kinase C modulators, calmodulin antagonists) and physiologic conditions (e.g., pH) [7,22,46,47], though it has been reported to be not saturable or inhibitable with structural analogue consistently with a transport via passive diffusion [8,21].

In conclusion, the results obtained in our cell system are consistent with the interpretation that impairment of intracellular drug accumulation, a common event in the development of cellular DDP resistance, is not mediated by CTR1 and CTR1 plays a marginal role in cellular pharmacology of cisplatin.

Acknowledgments

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