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ORIGINAL ARTICLE

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Cross-resistance to cisplatin in cells with acquired resistance to copper

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Abstract *Purpose*: Cells selected for resistance to cisplatin (DDP) demonstrate cross-resistance to copper (Cu) suggesting one or more common mechanisms of cellular defense. We sought to determine whether cells selected for resistance to Cu are cross-resistant to DDP. *Materials and methods*: Parental HuH7 human hepatoma cells and the CuR27 Cu-resistant subline were compared for sensitivity to Cu and DDP by clonogenic assay, and with respect to drug uptake and efflux by measuring cellular Cu and Pt content. *Results*: CuR27 cells were found to be 1.8-fold resistant to Cu and 8.6-fold cross-resistant to DDP. Changes in the cellular pharmacokinetics of Cu in the CuR27 cells were paralleled by changes in the kinetics of DDP. The accumulations of Cu and DDP measured at 1 min were, respectively, 36% and 26% of those in the parental HuH7 cells. The initial rate of efflux from the CuR27 cells was 6.2-fold faster for Cu and 2.5-fold faster for DDP than from the HuH7 cells. Cu reduced the accumulation of DDP in the HuH7 cells in a concentration-dependent manner and vice versa. DDP also reduced the efflux of Cu. Western blot analysis demonstrated that expression of the Cu exporter ATP7B was increased 3.9-fold in the CuR27 cells. *Conclusions*: In this model system, cross-resistance between Cu and DDP was bidirectional and accompanied by parallel changes in the cellular pharmacokinetics of both compounds. The results are consistent with the idea that transporters and chaperones that normally mediate Cu homeostasis also directly or indirectly modulate the accumulation of DDP.

Keywords Copper · Cisplatin · Resistance · Transport

Abbreviations *DDP* Cisplatin · *PBS* Phosphate-buffered saline · *TBS* Tris-buffered saline

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Introduction

Cells with acquired resistance to DDP characteristically accumulate lower levels of the drug [2, 11, 24]. Cellular accumulation of DDP is relatively slow compared to many other classes of chemotherapeutic agents and may involve both active and passive transporter-mediated processes [8, 9, 20]. Uptake is also dependent on temperature, pH [1, 4, 7], K^+ ion concentration [3] and reducing agents such as ascorbate and dithiothreitol [6, 29, 32]. Although the mechanisms for uptake and efflux of DDP are not well characterized, the behavior of this drug is similar in many ways to that of transition metals. DDP resistance is often associated with resistance to other metals and metalloids [27, 31]. DDP-resistant cells have been reported to be cross-resistant to arsenite [23, 27], antimony [5, 22, 23], cadmium [16, 26], zinc [15] and cobalt [22].

There is increasing evidence that DDP may enter and exit from cells utilizing transporters that have evolved for the management of Cu homeostasis. Cells selected for resistance to DDP are often cross-resistant to Cu [12]. The cellular pharmacokinetics of DDP and Cu have a number of common features. The uptake of both DDP and Cu requires the activity of the Na^+/K^+-ATPase [18, 21], apparently to maintain an appropriate gradient of K^+ ions [3, 25]. The efflux of both DDP and Cu is characterized by an initial rapid phase and a long secondary phase that is energy dependent [12, 19]. Ishida et al. [10] have reported that DDP uptake is impaired in yeast and mammalian cells in which the gene encoding the major Cu importer CTR1 has been deleted, and this

finding has been extended by Lin et al. [17] to show that CTR1 is important to the cellular uptake of all three clinically utilized platinum-containing drugs. Komatsu et al. [14] found that prostate cancer cells transfected with the Cu efflux pump ATP7B become resistant to DDP, and this has been confirmed in ovarian cancer models [13].

If transporters and chaperones that control Cu homeostasis play a central role in mediating resistance to DDP, then cells selected for acquired Cu resistance might be expected to exhibit cross-resistance to DDP. We report here that a human hepatoma cell line selected for resistance to Cu is in fact cross-resistant to DDP, and that the alterations in the cellular pharmacology of Cu in these cells are paralleled by changes in the kinetics of DDP. These results provide further confirmation of the linkage between cellular defense mechanisms for Cu and DDP and indicate that the transport of DDP into and out of the cell is influenced by components of the Cu homeostasis system.

Materials and methods

Drugs and reagents

Cupric sulfate, sodium arsenite, and other chemicals were obtained from Sigma (St. Louis, Mo.) and Fisher Scientific (Tustin, Calif.). DDP (PLATINOL-AQ) was a gift from Bristol Laboratories (Princeton, N.J.). The rabbit polyclonal antiserum for ATP7B was generously provided by Dr. J. Gitlin of Washington University (St. Louis, Mo.). Polyclonal antibody against β-actin was from Santa Cruz Biotechnology (Santa Cruz, Calif.). Secondary antibodies were purchased from Amersham Life Science (Piscataway, N.J.). Protein concentrations were measured using a protein assay kit from BIO-RAD (Richmond, Calif.). Western blotting reagents were purchased from BIO-RAD. Detection was made by film quantification of luminescence using an ECL kit from Amersham Life Science (Piscataway, N.J.).

Cells and colony assay

The human hepatocyte lines HuH7 and CuR27 were a gift from Dr. R. Stockert (The Liver Research Center, Albert Einstein College of Medicine, Bronx, N.Y.). They were grown in drug-free RPMI-1640 medium plus 10% fetal calf serum and maintained in humidified air containing 5% CO_2 at 37°C. Colony assays were performed using triplicate cultures of 200 cells per 35-mm plate grown in 5 ml medium containing different concentrations of DDP or cupric sulfate until visible colonies had formed (10–14 days). The dishes were rinsed twice with PBS, fixed with 100% methanol and stained with a 0.1% crystal violet solution. A ChemiImager 4400 instrument (Alpha Inotech, San Leandro, Calif.) was used for counting colonies of more than 50 cells.

Cellular pharmacokinetic assays

Uptake and efflux measurements were made using 100-mm tissue culture plates seeded with 10^6 cells each and incubated in medium until they were 75–80% confluent. Five plates were used for each data point. For uptake experiments, the medium was replaced by 10 ml fresh medium containing various concentrations of $CuSO_4$ or DDP and the cells were incubated at 37°C for various periods of time. Measurements of efflux rates were made by exposing the cells to DDP or $CuSO_4$ for 10 min, rinsing them with fresh medium, and incubating them for different times in drug-free medium at 37°C. At the requisite time point for both types of experiments, cultures were quickly rinsed three times with ice-cold PBS and cells were harvested into 15 ml ice-cold PBS using a rubber policeman. After centrifugation at 3000 rpm for 10 min the cells were resuspended in PBS, an aliquot was utilized for protein assay, and the remainder was digested in 68% nitric acid. Cell lysates were heated for 2 h at 65°C, diluted to 5% nitric acid and assayed for Pt and Cu content. A Perkin Elmer inductively coupled plasma optical emission spectroscopy apparatus (ICP-OES) model 3000DV from the Analytical Facility at the Scripps Institute of Oceanography was used for Cu and Pt assays.

Western blotting

Cells were rinsed twice with PBS, scraped with a rubber policeman, taken up in 10 ml PBS and centrifuged for 10 min at 2500 rpm. Cells were lysed in 0.25% Nonidet P-40 in 100 m*M* Tris HCl (pH 8.00) supplemented with 1 m*M* *p*-amidinophenyl methylsulfonyl fluoride hydrochloride and 1 m*M* γ-amino-*n*-caproic acid (Sigma, St. Louis, Mo.) at 4°C and for 30 min. Post-nuclear fractions were obtained by centrifugation of cell lysates for 10 min at 600 *g*. Samples of 50–100 µg protein content were electrophoresed on 4–10% SDS polyacrylamide gels. Gels were blotted onto nitrocellulose filters using a BIO-RAD Mini Transblot apparatus (BIO-RAD, Richmond, Calif.). Blots were incubated for 1 h with 5% fat-free dried milk in TBS at room temperature and then overnight in primary antibody mixed with 5% milk in TBS at 4°C. Blots were washed three times for 15 min each at room temperature with 0.025% Tween 20 in TBS. The secondary antibody was diluted in 5% fat-free dried milk in TBS and added to blots for 1 h at room temperature. Blots were washed again at room temperature three times for 15 min each in 0.025% Tween 20 in TBS. The extent of specific staining was quantified by chemiluminescence using the ECL kit from Amersham Life Sciences (Piscataway, N.J.). A ChemiImager 400 instrument (Alpha Inotech, San Leandro, Calif.) was used for determining the density of protein bands.

Statistics

Tests of significance utilized Student's *t*-test; *P* values ≤ 0.05 were considered significant.

Results

Cross-resistance between Cu and DDP

The Cu-resistant subline CuR27 was generated from the parental HuH7 human hepatoma line by repeated in vitro exposure to $CuSO_4$ by Schilsky et al. [30]. The sensitivity of these cells to Cu and DDP was determined by clonogenic assay using continuous drug exposure.

Table 1 Cross-resistance between DDP and $CuSO_4$. The IC_{50} values are the means of assays performed with triplicate cultures

Cell type	DDP		Cupric sulfate	
	IC_{50} (µ*M*)[a]	Fold resistance	IC_{50} (µ*M*)[a]	Fold resistance
HuH7	0.03	–	38.7	–
CuR27	0.24	8.6	70.6	1.8

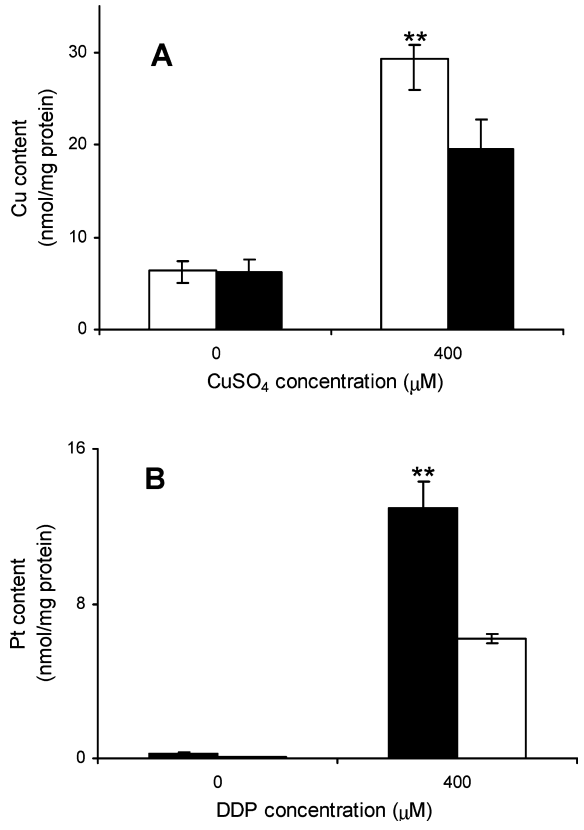

Fig. 1A, B Cu and Pt accumulation in parental HuH7 and Cu-resistant CuR27 cells following a 1-h exposure: **A** Cells exposed to $CuSO_4$; **B** cells exposure to DDP (*open bars* HuH7 cells, *filled bars* CuR27 cells). Each data point represents the mean of three independent measurements (*vertical bars* ± SEM). $^{**}P \leq 0.0001$

The IC_{50} values presented in Table 1 indicate that the CuR27 cells were 1.8-fold resistant to Cu, but fully 8.6-fold cross-resistant to DDP. Thus, selection for relatively low-level resistance to Cu resulted in a much higher level of resistance to DDP. We have previously observed the same phenomenon in the opposite direction with cells selected for DDP resistance exhibiting a greater degree of resistance to DDP than cross-resistance to Cu [12].

Cellular accumulation of DDP and Cu at 1 h

The ability of HuH7 and CuR27 cells to accumulate Cu and DDP was assessed by exposing the cells for 1 h, washing them extensively, and then measuring cell-associated Cu and Pt content. Figure 1 shows that the net amount of Cu accumulated by the CuR27 Cu-resistant subline was lower than that accumulated by the parental HuH7 cells measured at the end of a 1-h exposure to 400 μ*M* $CuSO_4$. The amount of Cu associated with the CuR27 cells was 58 ± 4% (mean ± SEM) of that associated with the parental HuH7 cells. This result is consistent with previously published data on the same cell line [30]. The amount of Pt accumulated by the CuR27 cells after exposure to 400 μ*M* DDP for 1 h was

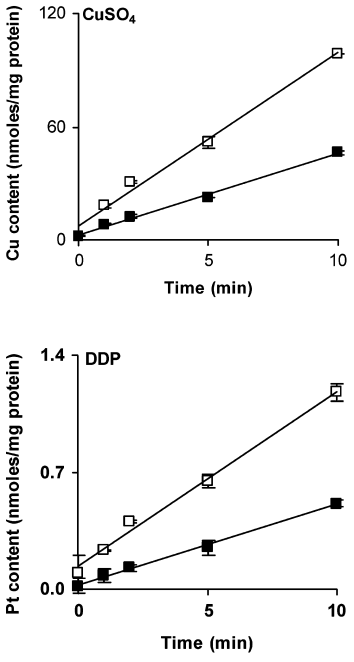

Fig. 2 Linearity of Cu and DDP accumulation in HuH7 and CuR27 cells. HuH7 (□) and CuR27 (■) cells were exposed to 400 μ*M* $CuSO_4$ or DDP. Each data point represents the mean of five independent measurements (*vertical bars* ± SEM)

51 ± 2% of that accumulated by the HuH7 cells. These statistically significant reductions ($P < 0.0001$) indicate that the alterations in the mechanisms of cellular Cu accumulation that occurred during the emergence of Cu resistance in the CuR27 cells were accompanied by events that produced parallel changes in the net accumulation of DDP.

Initial accumulation rate

The levels of accumulation at 1 h reflect the net contribution of both influx and efflux and differences in cell surface and intracellular binding. Ideally, one would like to measure the initial influx rate to assess the extent to which a reduction in the ability of the drug to enter the cell contributes to the difference in accumulation observed at 1 h. Cu and DDP both enter cells quite slowly, and the shortest time interval over which a reliable increase in cell-associated Cu and DDP could be detected in these cells during exposure to 400 μ*M* drug was 1 min. As shown in Fig. 2, accumulation of both Cu and DDP was linear over the first 10 min indicating that measurements made at 1 min are a reasonable estimate of the initial accumulation rate.

The values for the 1-min accumulation of Cu and Pt in the HuH7 and CuR27 cells are presented in Table 2. The accumulation of Cu in the CuR27 cells was 43 ± 3% (mean ± SEM) of that for the parental HuH7 cells ($P < 0.01$). The accumulation of DDP in the CuR27 cells was only 26 ± 1% of that in the HuH7 cells ($P < 0.02$). A direct comparison of the 1-min accumulation for Cu

Table 2 Cu and DDP accumulation rates measured over the first minute of exposure. Values are means ± SEM of five independent measurements

Cell type	Accumulation rate (pmol/mg protein/min)	
	Cu	DDP
HuH7	15,270.4 ± 1059.2	316.5 ± 55.9
CuR27	6,510.2 ± 382.9	81.6 ± 3.5
	$P \leq 0.01$	$P \leq 0.02$

versus DDP in the parental HuH7 cells demonstrated that the basal rate of accumulation of DDP was 2.0 ± 0.4% of that for Cu ($P < 0.008$). Thus, while the absolute rate of DDP accumulation over the first minute was much lower than that for Cu, the magnitude of the decrease in accumulation observed in the Cu-resistant cells was similar for both Cu and DDP.

Efflux of Cu and Pt

The efflux of Cu and Pt from HuH7 and CuR27 cells was examined following loading of the cells with Cu or DDP by exposing them for 10 min to 400 μ*M* of each compound. As shown in Fig. 3A, relatively little of the Cu and DDP associated with the cells at 10 min was available for efflux. In the case of the HuH7 cells, only 64 ± 13% of the Cu and only 23 ± 2% of the Pt had exited the cells after 120 min. The fraction of Cu and Pt that was available for efflux left the cells rapidly. Additional experiments were performed to better define the initial phase of efflux and these data were fitted to a two-compartment pharmacokinetic model as shown in Fig. 3A. This yielded an accurate estimate of the initial half-life, but because the terminal half-life was so very slow it was not possible to develop a reliable estimate from the data available. As shown in Table 3, the estimated initial half-life for Cu efflux from the CuR27 cells was 6.2-fold shorter than from the HuH7 cells. A somewhat smaller difference was observed for Pt efflux for which the initial half-life was 2.5-fold shorter for the CuR27 than for the HuH7 cells. Thus, development of Cu resistance in the CuR27 cells was accompanied by a substantial increase in the rate at which the rapidly effluxable fraction of the compound exited from the cell, and the changes in initial Cu efflux were accompanied by similar changes in the initial phase of Pt efflux.

Table 3 Initial half-lives of Cu and DDP from HuH7 and CuR27 cells (*CV* coefficient of variation of half-life estimate)

Cell line	Cu		DDP	
	$t_{1/2\alpha}$ (min)	CV (%)	$t_{1/2\alpha}$ (min)	CV(%)
HuH7	0.98	50	0.61	16
CuR27	0.16	20	0.24	18

Interaction between DDP and Cu during accumulation

The results reported above are consistent with the concept that Cu and DDP have some uptake and efflux mechanisms in common, or interact with common binding sites on or in the cell. This possibility was

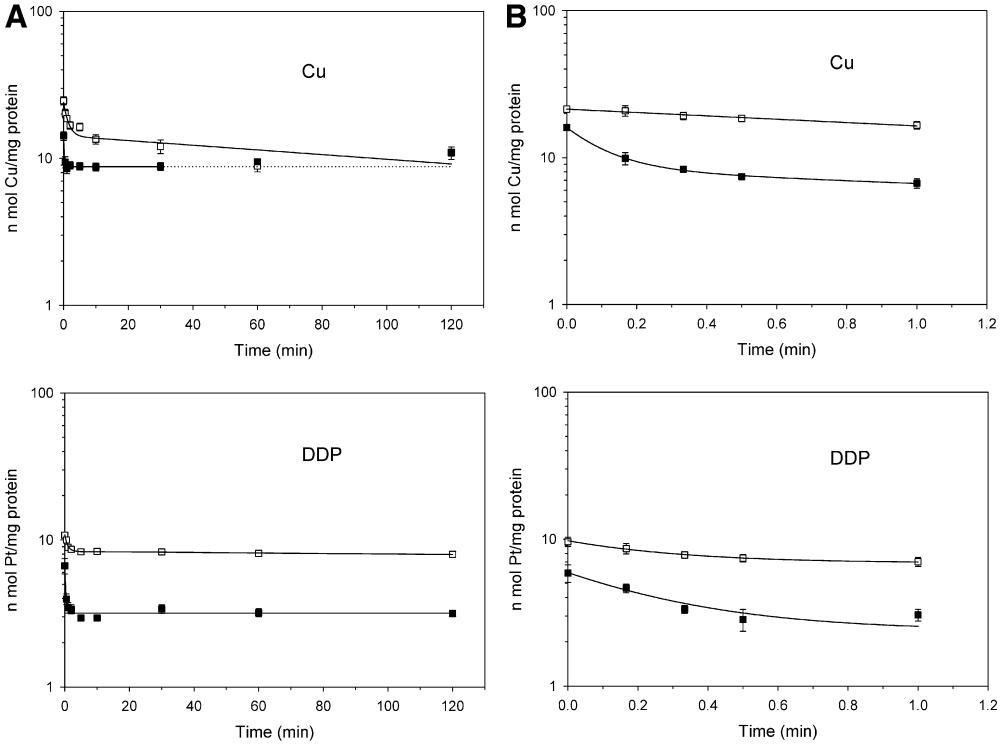

Fig. 3A, B Efflux of Cu and DDP from parental HuH7 and Cu-resistant CuR27 cells. HuH7 (□) and CuR27 (■) cells were loaded by exposure to 400 μ*M* $CuSO_4$ or DDP for 10 min: **A** efflux over 120 min; **B** efflux over 60 s. Each data point represents the mean of five independent measurements (*vertical bars* ± SEM)

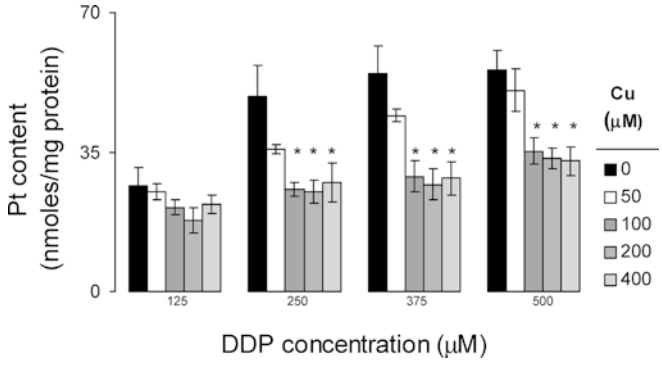

Fig. 4 Effect of Cu on DDP accumulation in HuH7 cells. Each bar depicts the amount of Cu in the HuH7 cells after a 10-min exposure to the indicated concentration of $CuSO_4$ in the absence of DDP or in the presence of increasing concentrations of DDP. Each data point represents the mean of five independent measurements (*vertical bars* ±SEM). vs Cu content of cells exposed to Cu alone. $^*P < 0.05$

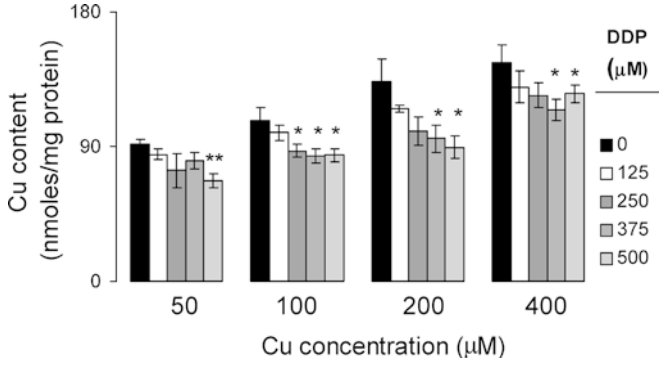

Fig. 5 Effect of DDP on Cu accumulation in HuH7 cells. Each bar depicts the amount of Pt in the HuH7 cells after a 10-min exposure to the indicated concentration of DDP in the absence of $CuSO_4$ or in the presence of increasing concentrations of $CuSO_4$. Each data point represents the mean of five independent measurements (*vertical bars* ±SEM). vs Cu content of cells exposed to Cu alone. $^*P < 0.05$

explored more directly by examining the effect of increasing concentrations of Cu on the accumulation of DDP and vice versa. As accumulation of both Cu and DDP was linear over the first 10 min, accumulation was measured at this time point in order to permit more accurate measurements than could be made using 1-min drug exposures. As shown in Fig. 4, the amount of Pt associated with the cells increased with increasing extracellular DDP concentration in the absence of Cu. When Cu was present, it reduced the amount of cell-associated DDP. For any given DDP concentration, as the extracellular $CuSO_4$ concentration was increased there was a progressive decrease in the amount of DDP associated with the cells at all DDP concentrations tested over the range of 50–400 μ*M*. Figure 5 shows that DDP also altered the net accumulation of Cu. As the DDP concentration was increased in the presence of a given extracellular concentration of $CuSO_4$, there was a progressive decrease in the extent of cell-associated Cu

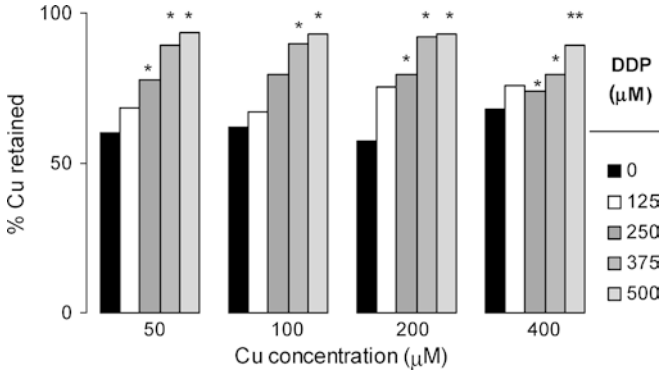

Fig. 6 Effect of DDP on Cu efflux from HuH7 cells. Each bar depicts the percent of Cu remaining in the cells 30 s after the start of efflux and represents the mean of five independent measurements. vs percent of Cu remaining in the absence of DDP exposure. $^*P < 0.05$

at all $CuSO_4$ concentrations tested. These results demonstrate that each compound is capable of altering the accumulation of the other either through competition for a transport process or alteration of the structure or function of a transporter or other molecules that bind these compounds. Since available instrumentation limited the accuracy of true initial influx rate measurements, the nature of the interaction between DDP and Cu could not be reliably further assessed.

Effect of DDP on Cu efflux

The effect of increasing amounts of intracellular DDP on the initial efflux of Cu was measured by loading HuH7 cells with a 10-min exposure to a given concentration of $CuSO_4$ alone or in the presence of increasing concentrations of DDP, and then measuring the fraction of Cu remaining in the cells 30 s after the extracellular Cu and DDP were removed using a rapid sampling technique. The $CuSO_4$ concentration used for loading was varied over the range of 0–400 μ*M*. The data presented in Fig. 6 indicate that, in the absence of concurrent loading with DDP, the fraction of Cu that remained in the cells 30 s after the start of efflux did not vary as a function of the concentration of $CuSO_4$ used to load the cells. However, at all $CuSO_4$ concentrations tested, the concurrent presence of DDP during the loading process caused a reduction in Cu efflux, and this effect increased with increasing DDP concentration. The data do not permit a formal analysis of the nature of the interaction between Cu and DDP with respect to efflux because the actual intracellular concentrations of the free substrates are not known.

ATP7B expression

It has previously been reported that ATP7B mRNA and protein levels are increased in the Cu-resistant CuR27

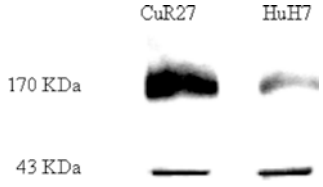

Fig. 7 Western blot analysis of ATP7B protein expression in CuR27 and HuH7 cells (*upper panel*). Lysates containing 50 μg of total cellular protein were analyzed with a polyclonal antiserum against the ATP7B. The *lower panel* shows the same blot probed for the expression of β-actin

cells relative to the levels found in the parental HuH7 cells [30]. Because of its potential to affect the cellular pharmacokinetics of Cu, the expression of ATP7B was re-examined by Western blotting. As shown in Fig. 7, the CuR27 cells had 3.9-fold more ATP7B protein than the HuH7 cells.

Discussion

Cu and DDP are believed to kill cells by different mechanisms. Excess Cu generates reactive oxygen species that have the potential to damage many components of the cellular machinery, whereas the cytotoxic effect of DDP is mediated largely through the formation of adducts in DNA. Despite this difference, the results reported here provide evidence that the mechanisms that protect cells against Cu also protect against the toxicity of DDP. The observation that the CuR27 cells selected for low-level Cu resistance demonstrate a much higher level of resistance to DDP suggests that DDP may be quite dependent on the transporters and chaperones that normally mediate Cu homeostasis for access to the cell or critical targets in the cell. The changes in Cu accumulation and efflux found in the Cu-resistant CuR27 cells were accompanied by similar changes in the cellular pharmacology of DDP. Furthermore, DDP was found to alter both the accumulation and efflux of Cu. DDP can still enter cells slowly in the absence of the major Cu influx transporter CTR1 [10, 17], so this drug has a route of entry that may be unrelated to a Cu transporter. However, these observations indicate the influx of DDP, its intracellular binding, or its export from the cell is controlled by Cu transporters and/or chaperones to a degree sufficient to mediate clinically significant degrees of resistance. It is important to emphasize that the effects of the Cu homeostasis proteins could be indirect rather than direct since the activity of Cu-requiring enzymes could secondarily influence detoxification mechanisms unrelated to Cu transporters or chaperones.

Under conditions of continuous exposure, the CuR27 cells were found to be 1.8-fold resistant to Cu and 8.6-fold resistant to DDP. When measured with this type of assay, this constitutes a high level of resistance to DDP. While detailed cellular pharmacology studies have been performed only in the HuH7 and CuR27 cell pair, we have also observed cross-resistance to DDP in CuR23 cells, another variant of the HuH7 line selected for Cu resistance. The CuR23 cells were 1.5-fold resistant to Cu and 8.9-fold resistant to DDP; detailed cellular pharmacology studies have not been done with the CuR23 cells [28]. Cross-resistance to Cu has also been found in cells selected for resistance to DDP. In studies performed with four human tumor cell lines, we have found that cells selected for resistance to DDP are cross-resistant to Cu, and that the magnitude of the resistance is consistently lower for Cu than for DDP [12]. It would be of interest in the future to isolate a DDP-resistant variant of the HuH7 hepatoma line so that the Cu- and DDP-resistant variants could be directly compared in the same cell system. Nevertheless, the fact that the CuR23 and CuR27 cells selected for resistance to Cu are cross-resistant to DDP and vice versa already provides strong evidence for common cellular defense mechanisms for these compounds.

The development of Cu resistance in the CuR27 cells was accompanied by reduced Cu accumulation and increased ATP7B expression. The Cu resistance of these cells has previously been ascribed to increased expression of this Cu export protein [30]. Consistent with the ability of ATP7B to function as a Cu exporter, Cu efflux was more rapid from these cells than from the parental HuH7 cells. Two phases of efflux could be detected: a quite rapid phase during which only a small fraction of the Cu present in the cell was exported, and a very much slower phase during which very little additional Cu was lost from the cells. A similar pattern was observed for efflux of Pt. Although fitting to a two-compartment model yielded a reasonable estimate of the initial efflux half-lives, the terminal rate could not be accurately defined because it was so very slow. Cu and Pt efflux during the initial phase was clearly increased in the CuR27 cells. It is important to note that the differences in efflux may be due to variation in the extent of intracellular binding or subcellular sequestration rather than to differences in the actual efflux rate constants. Likewise, the lack of good correspondence between the differences in accumulation and efflux and the magnitude of the resistance to Cu relative to DDP suggests that other cellular pharmacokinetic parameters, such as rate of delivery of metalloid from the cytoplasm to DNA, may also contribute importantly to resistance to DDP.

Irrespective of whether the Cu-resistant phenotype of the CuR27 cells is due solely to decreased accumulation or increased Cu efflux, or whether changes in additional Cu homeostatic proteins are also involved, it is remarkable that the changes in Cu accumulation and efflux were so closely paralleled by similar changes in the cellular pharmacokinetics of DDP. The accumulation of Cu at 1 min in the CuR27 cells was only 42% of that in the parental cells; the accumulation of DDP in the CuR27 cells was 26% of that in the parental cells. The initial Cu efflux rate was increased by 6.2-fold in the CuR27 cells; the DDP efflux rate was increased by 2.5-fold in these cells. Since the initial efflux of Cu is so

rapid, it is possible that the deficit in 1-min accumulation may simply reflect the increased ATP7B activity in the CuR27 cells. On the other hand, altered activity of the influx transporter may also contribute to Cu resistance in the CuR27 cells. CTR1 is the major Cu influx transporter in human cells; however, no antibodies are commercially available to this protein, and its level in the CuR27 cells cannot be quantified at this time. Nevertheless, the current observations suggest that transporters or intracellular binding proteins responsible for the changes in Cu cellular pharmacology in the CuR27 cells are also responsible for the changes in DDP accumulation and efflux.

A possible role for ATP7B in the export of DDP has previously been suggested [14] and confirmed in studies from this laboratory [13]. Further evidence for this concept is provided by the observation that Cu blocks the accumulation of DDP and vice versa in the parental HuH7 cells, and the finding that DDP reduces the efflux of Cu from the cell. However, these findings cannot be interpreted to indicate true competition for transport. Because both Cu and DDP have the potential of binding to the metal binding sequences found in these proteins, the effects may be due to altered structure of the Cu transporters rather than competition for entrance into a transmembrane channel. This renders the assessment of the actual nature of the interaction between DDP and Cu difficult. This is particularly true for the efflux studies because there is no practical way of measuring the actual free intracellular Cu or DDP concentration inside the cell.

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References

1. Amtmann E, Zoller M, Wesch H, Schilling G (2001) Antitumoral activity of a sulphur-containing platinum complex with an acidic pH optimum. Cancer Chemother Pharmacol 47:461
2. Andrews PA, Howell SB (1990) Cellular pharmacology of cisplatin: perspectives on mechanisms of acquired resistance. Cancer Cells 2:35
3. Andrews PA, Mann SC, Huynh HH, Albright KD (1991) Role of the Na+, K+-ATPase in the accumulation of cis-diammine-dichloroplatinum(II) in human ovarian carcinoma cells. Cancer Res 51:3677
4. Atema A, Buurman KJ, Noteboom E, Smets LA (1993) Potentiation of DNA-adduct formation and cytotoxicity of platinum-containing drugs by low pH. Int J Cancer 54:166
5. Chen ZS, Mutoh M, Sumizawa T, Furukawa T, Haraguchi M, Tani A, Saijo N, Kondo T, Akiyama S (1998) An active efflux system for heavy metals in cisplatin-resistant human KB carcinoma cells. Exp Cell Res 240:312
6. Chiang CD, Song EJ, Yang VC, Chao CC (1994) Ascorbic acid increases drug accumulation and reverses vincristine resistance of human non-small-cell lung-cancer cells. Biochem J 301:759
7. Endo T, Kimura O, Sakata M (2000) Carrier-mediated uptake of cisplatin by the OK renal epithelial cell line. Toxicology 146:187
8. Gately DP, Howell SB (1993) Cellular accumulation of the anticancer agent cisplatin: a review. Br J Cancer 67:1171
9. Hromas RA, North JA, Burns CP (1987) Decreased cisplatin uptake and binding as a mechanism of resistance in L1210 leukemia cells. Cancer Lett 36:197
10. Ishida S, Lee J, Thiele DJ, Herskowitz I (2002) Uptake of the anticancer drug cisplatin mediated by the copper transporter Ctr1 in yeast and mammals. Proc Natl Acad Sci U S A 99:14298
11. Kartalou M, Essigmann JM (2001) Mechanisms of resistance to cisplatin. Mutat Res 478:23
12. Katano K, Kondo A, Safaei R, Holzer A, Samimi G, Mishima M, Kuo Y-M, Rochdi M, Howell S (2002) Acquisition of resistance to cisplatin is accompanied by changes in the cellular pharmacology of copper. Cancer Res 62:6559
13. Katano K, Safaei R, Samimi G, Holzer A, Rochdi M, Howell SB (2003) The copper export pump ATP7B modulates the cellular pharmacology of carboplatin in ovarian carcinoma cells. Mol Pharmacol 64:466
14. Komatsu M, Sumizawa T, Mutoh M, Chen Z-S, Terada K, Furukawa T, Yang X-L, Gao H, Miura N, Sugiyama T, Akiyama S (2000) Copper-transporting P-type adenosine triphosphatase (ATP7B) is associated with cisplatin resistance. Cancer Res 60:1312
15. Koropatnick J, Pearson J (1990) Zinc treatment, metallothionein expression, and resistance to cisplatin in mouse melanoma cells. Somat Cell Mol Genet 16:529
16. Lee KB, Parker RJ, Reed E (1995) Effect of cadmium on human ovarian cancer cells with acquired cisplatin resistance. Cancer Lett 88:57
17. Lin X, Okuda T, Holzer A, Howell SB (2002) The copper transporter CTR1 regulates cisplatin uptake in Saccharomyces cerevisiae. Mol Pharmacol 62:1154
18. Ma J, Maliepaard M, Kolker HJ, Verweij J, Schellens JH (1998) Abrogated energy-dependent uptake of cisplatin in a cisplatin-resistant subline of the human ovarian cancer cell line IGROV-1. Cancer Chemother Pharmacol 41:186
19. Mann SC, Andrews PA, Howell SB (1990) Short-term cis-diamminedichloroplatinum (II) accumulation in sensitive and resistant human ovarian carcinoma cells. Cancer Chemother Pharmacol 25:236
20. Mann SC, Andrews PA, Howell SB (1991) Modulation of cis-diamminedichloroplatinum(II) accumulation and sensitivity by forskolin and 3-isobutyl-1-methylxanthine in sensitive and resistant human ovarian carcinoma cells. Int J Cancer 48:866
21. Murphy AS, Eisinger WR, Shaff JE, Kochian LV, Taiz L (1999) Early copper-induced leakage of K(+) from Arabidopsis seedlings is mediated by ion channels and coupled to citrate efflux. Plant Physiol 121:1375
22. Naredi P, Heath DD, Enns RE, Howell SB (1994) Cross-resistance between cisplatin and antimony in a human ovarian carcinoma cell line. Cancer Res 54:6464
23. Naredi P, Heath DD, Enns RE, Howell SB (1995) Cross-resistance between cisplatin, antimony potassium tartrate, and arsenite in human tumor cells. J Clin Invest 95:1193
24. Parker RJ, Eastman A, Bostick-Bruton F, Reed E (1991) Acquired cisplatin resistance in human ovarian cancer cells is associated with enhanced repair of cisplatin-DNA lesions and reduced drug accumulation. J Clin Invest 87:772
25. Pena MM, Lee J, Thiele DJ (1999) A delicate balance: homeostatic control of copper uptake and distribution. J Nutr 129:1251
26. Perego P, Vande Weghe J, Ow DW, Howell SB (1997) Role of determinants of cadmium sensitivity in the tolerance of Schizosaccharomyces pombe to cisplatin. Mol Pharmacol 51:12
27. Romach EH, Zhao CQ, Del Razo LM, Cebrian ME, Waalkes MP (2000) Studies on the mechanisms of arsenic-induced self tolerance developed in liver epithelial cells through continuous low-level arsenite exposure. Toxicol Sci 54:500

28. Safaei R, Howell S (2001) Cross-resistance between cisplatin, carboplatin, copper, and arsenite in human tumor cell lines. Proc Am Assoc Cancer Res 42:424
29. Sarna S, Bhola RK (1993) Chemo-immunotherapeutical studies on Dalton's lymphoma in mice using cisplatin and ascorbic acid: synergistic antitumor effect in vivo and in vitro. Arch Immunol Ther Exp 41:327
30. Schilsky ML, Stockert RJ, Kesner A, Gorla GR, Gagliardi GS, Terada K, Miura N, Czaja MJ (1998) Copper resistant human hepatoblastoma mutant cell lines without metallothionein induction overexpress ATP7B. Hepatology 28:1347
31. Tobey RA, Tesmer JG (1985) Differential response of cultured human normal and tumor cells to trace element-induced resistance to the alkylating agent melphalan. Cancer Res 45:2567
32. Zhang JG, Zhong LF, Zhang M, Ma XL, Xia YX, Lindup WE (1994) Amelioration of cisplatin toxicity in rat renal cortical slices by dithiothreitol in vitro. Hum Exp Toxicol 13:89