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Accumulation, platinum–DNA adduct formation and cytotoxicity of cisplatin, oxaliplatin and satraplatin in sensitive and resistant human osteosarcoma cell lines, characterized by p53 wild-type status

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ABSTRACT

p53 gene status is implicated in the cytotoxic drug sensitivity and published research has been mostly addressed to cisplatin (CDDP) activity. Previous study in our laboratory considered p53 mutant cell lines A431 (parental) and A431/Pt (CDDP-resistant counterpart, resistance factor R.F. = 2.6). For a comparison which contributes to a deeper appreciation of the process that mediates the Pt drug cellular effects, we extended our investigation to the p53 wild-type cell lines U2-OS (human osteosarcoma) and its CDDP-resistant counterpart U2-OS/Pt (R.F. = 5). We compared the activity of CDDP, oxaliplatin (L-OHP) and satraplatin (JM216) whose hydrophobicity rank is JM216 > L-OHP > CDDP.

In U2-OS cells the three drugs accumulated similarly, while in U2-OS/Pt the most hydrophobic drugs were privileged. No significant differences in efflux were observed between sensitive and resistant cell lines. The growing of CDDP resistance seems to be overcome by increasing the hydrophobicity of the Pt agent. An almost linear trend seems to relate R.F. and drug hydrophobicity in U2-OS/Pt and A431/Pt cells. DNA platination in U2-OS as in A431 cells is at the lowest levels for L-OHP. In U2-OS cell line the IC_{50} of CDDP (17.6 μM) and JM216 (88.02 μM) do not correlate with their similar levels of Pt–DNA adducts (mean value ~0.14 pmol Pt/μg DNA). The presence of a wild-type p53 exalts either CDDP cytotoxicity (two-fold more active in U2-OS than in A431 cells) and CDDP resistance in comparison to a p53 mutant type. The p53 status seems to not improve JM216 or L-OHP cytotoxicity in both cell lines.

1. Introduction

The resistance to cisplatin (CDDP) is multifactorial and several mechanisms are encountered simultaneously within the same tumour cell [1]. The high level of resistance is usually an effect of a set of almost unrelated mechanisms [2]. Cells expressing low levels of CDDP resistance, that mimic the clinical situation [3], should be more prone to

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show a relatively simplified cellular effect scenario, at least considering the resistance. Previously, we studied the cell system A431–A431/Pt (sensitive and CDDP-resistant human cervical tumour cells) where the resistant cells were characterized by a low level of CDDP resistance (resistance factor, R.F. = 2.6) [4]. Administering to this cell model CDDP, oxaliplatin (L-OHP) and satraplatin (JM216), whose hydrophobicity is in the order JM216 > L-OHP > CDDP [5], we showed that in the resistant cells the reduction of CDDP accumulation appeared as one of the major determinants of CDDP resistance, and variation(s) of plasma membrane accounted for this reduction [4]. The same model system was chosen by Lanzi et al. [6] to evaluate the role of p53 gene status in acquired resistance to CDDP. They proved that the limited sensitivity of the parental cell line to CDDP exposure and the low level of drug-induced apoptosis were consistent with the presence of a mutant p53 gene [6]. p53 is involved in the mediation of the CDDP toxic cellular events which are a direct consequence of both DNA damage and of the amount of Pt–DNA adducts [7]. p53 protein, a transcription factor acting as tumour suppressor, seems to sense the extent of DNA damage and, thereby, determines whether to permit the cell to survive or activate the apoptotic program [7]. Therefore, tumour cells that have gene mutations in the apoptotic function of p53 fail to activate the cell death program [7]. On the contrary, the presence of wild-type p53 facilitates CDDP cytotoxicity as happens for instance in seminomatous germ cell tumour [8]. Many of the studies defining the role of p53 in the apoptotic pathway have been conducted administering CDDP, but not its analogues, to cells [7,9]. Recently the role of p53 in response to different types of DNA damage induced by CDDP and BBR3464 in U2-OS human osteosarcoma cell line has been considered and only CDDP was capable of inducing significant levels of apoptosis [10]. Furthermore, JM216-, JM118- and CDDP-induced cytotoxicities in relation to Pt–DNA adduct formation do not appear related to p53 status of the considered cell lines [11]. We have been currently evaluating cell sensitivity, cellular accumulation and Pt–DNA adducts of CDDP, L-OHP and JM216, characterized by different hydrophobicities [5], in pairs of sensitive and CDDP-resistant cells with low R.F. This information can highlight the role of p53 in cellular response to drugs different from CDDP, hopefully leading to criteria for a more appropriate choice of Pt-drug also in clinical situation. So, we extended our study to U2-OS and U2-OS/Pt (parental and its CDDP-resistant counterpart, respectively). This model presents a p53 wild-type status [10,12], well-defined biological features and molecular characteristics [13] and the reduced drug accumulation has been shown to be an important feature in the setting up of the five-fold CDDP-resistance [14].

The present work on the pair U2-OS and U2-OS/Pt was intended (i) to evaluate the role of p53 wild-type status in determining the cytotoxicity in relation to Pt–DNA adducts of the different drugs; (ii) to check if membrane modification(s) were still present and contributed to the CDDP-resistance; (iii) to test the importance of the drug hydrophobicity in overcoming a doubled R.F. in comparison to A431/Pt.

2. Material and methods

2.1. Cell lines and culture conditions

The human tibial osteosarcoma cell line U2-OS and the CDDP-resistant counterpart U2-OS/Pt were used in this study [13]. Establishment details and biological properties have been already described [13]. Both cell lines grew as monolayers at 37 °C in a 5% CO_2 atmosphere in Mc Coy's 5A medium (BioWhittaker Italia s.r.l., Milano, Italy) supplemented with 10% heat inactivated FCS (Gibco Srl, Milano, Italy) and 2 mM L-glutamine (Sigma–Aldrich S.r.L., Milano, Italy). Antibiotics were omitted from the medium to avoid interactions. The parental and the resistant lines were used from passage 2 to 20. Both cell lines were kindly provided by Dr. Paola Perego, Department of Experimental Oncology and Laboratories, Istituto Nazionale Tumori, Milano, Italy.

2.2. Platinum drugs

CDDP (Platamine®; Pharmacia & Upjohn S.p.A., Milano, Italy); L-OHP (Eloxatine®) was a gift from Sanofi-Synthelabo S.p.A., Milano, Italy; JM216 was provided by one of us (L.K.). Immediately before use, CDDP and JM216 were dissolved in 0.9% saline, while L-OHP was dissolved in water.

2.3. Assessment of cellular sensitivity to drugs

Cellular sensitivity to drugs was assessed by cell counting accordingly to our previous work [4]. All assays were performed in triplicate, and the values are the mean ± standard deviation (S.D.) of three independent experiments. IC_{50} determined by extrapolation of dose–response cytotoxic curves is defined as the concentration resulting in 50% reduction in cell number as compared to untreated plates. The R.F. was calculated as

$$\text{R.F.} = \frac{IC_{50}\text{U2-OS/Pt cells}}{IC_{50}\text{U2-OS cells}}$$

2.4. Determination of cellular Pt accumulation

Cellular Pt accumulation was determined for both cell lines, exposed for 1 h to freshly dissolved CDDP, L-OHP or JM216 at concentrations up to 500 μM. The method was according standard procedures [4,15]; total Pt content (detection limit 10 μg/l) was determined by flameless absorption spectroscopy (Model 3300, Perkin-Elmer, Norwalk, CT) at the Institute of Occupational Medicine, University of Brescia, Italy. Experiments were performed in duplicate and the values are the means ± S.D. of three independent experiments.

2.5. Drug efflux studies

Both cell lines were seeded as described above for cellular Pt accumulation studies. Twenty-four hours later, the U2-OS and U2-OS/Pt cells were treated with 75 and 400 μM CDDP, respectively to obtain, after 60 min, similar cellular Pt levels in the two cell lines (see Pt accumulation). Details of the method have been previously published [4]. Experiments were

performed in duplicate and the values are the means ± S.D. of three independent experiments.

2.6. Quantification of Pt–DNA adducts

For both cell lines, 4.5×10^6 cells were seeded in 100 mm tissue culture dishes (Iwaki). After 24 h, the cells were exposed for 1 h to CDDP, L-OHP or JM216 (as described previously for Pt accumulation) at concentrations ranging up to 500 μM. Lysis of cells was carried out overnight at 37 °C accordingly to [4]. DNA was extracted, using a combined phenol–Tris HCl (Labtek S.r.L Eurobio, Milano, Italy) and chloroform–ethanol–sodium acetate (Sigma) technique (modification of the method of Kirby and Cook [16]). The amount and purity of DNA were determined according to [17]. Pt content of the samples was assessed by inductively coupled plasma mass spectroscopy [15] at the Institute of Occupational Medicine, University of Brescia, Italy. DNA platination levels (detection limit 0.1 μg/l) were expressed as pmol Pt/μg DNA. Experiments were made in duplicate and the values are the means ± S.D. of three independent experiments.

2.7. Statistical analysis

Data of Pt uptake, adducts and Pt efflux were plotted using a linear regression model. For all the plots a significant regression coefficient was found ($p < 0.01$ or less).

Statistical significance of the regression was evaluated by using unpaired two-tailed Student's *t*-test. Difference between linear regressions was obtained by comparing the slope with Student's *t*-test. A critical value of $p < 0.05$ was considered.

3. Results

3.1. Assessment of cellular sensitivity to Pt drugs

Cellular sensitivity to Pt drugs was determined by growth-inhibition assay after 1 h drug exposure. The IC_{50} values are shown in Table 1. Under our experimental conditions, the U2-OS/Pt cell line was approximately five- and two-fold more resistant to CDDP and L-OHP, respectively. No significant differences in IC_{50} values were observed when the two cell lines were exposed to JM216. The IC_{50} values of U2-OS cells were approximately 6.8-fold and 5-fold higher for L-OHP and JM216, respectively, than CDDP.

Table 1 – Cell sensitivity to CDDP, L-OHP and JM216 in U2-OS and U2-OS/Pt cells[a]

Drugs	IC_{50} (μM)		R.F.[b]
	U2-OS	U2-OS/Pt	
CDDP	17.6 ± 4.8	87.4 ± 4.2	4.96
L-OHP	120.2 ± 3.5	245.8 ± 3.5	2.04
JM216	88.0 ± 8.3	94.4 ± 3.3	1.07

[a] Cell sensitivity was assessed in cells exposed for 1 h to the drug and counted 72 h later.
[b] R.F. = resistance factor, calculated as: R.F. = (IC_{50}U2-OS/Pt cells)/(IC_{50}U2-OS cells).

3.2. Determination of cellular Pt accumulation

Total cellular Pt levels after 1 h drug exposure (range 35–500 μM) are expressed in terms of nmol Pt/10^6 cells, and are shown in

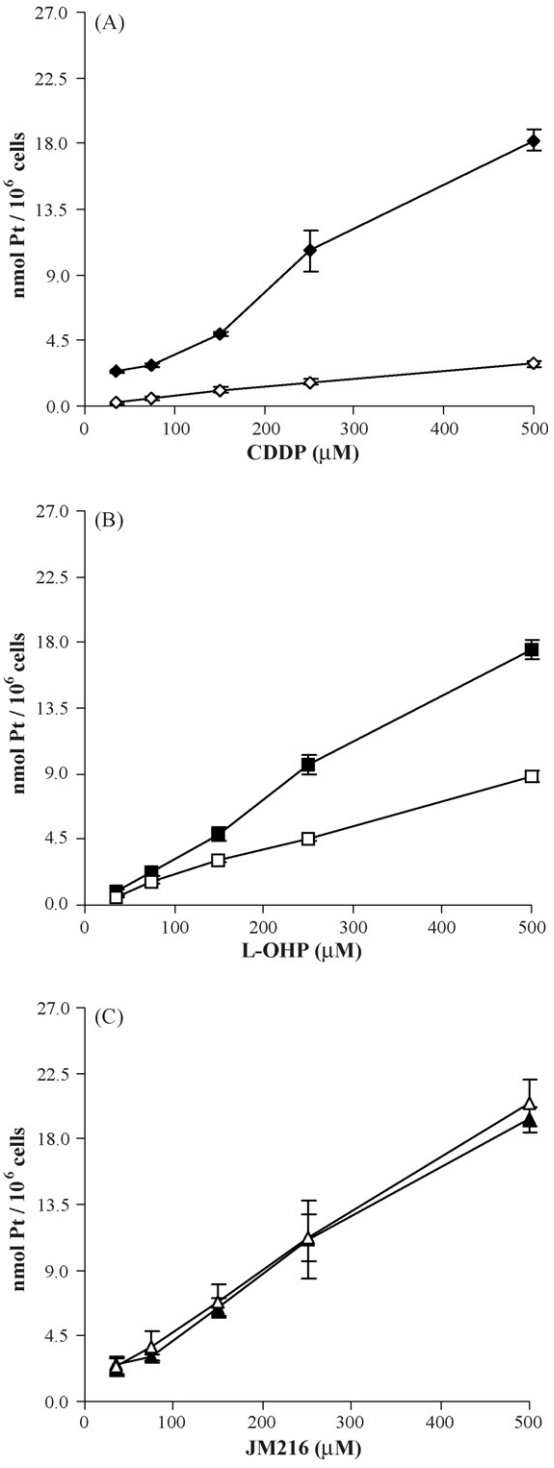

Fig. 1 – Pt accumulation after 1 h exposure to increasing concentrations of (A) CDDP in the U2-OS (◆) and in the U2-OS/Pt (◇) cell lines; (B) L-OHP in the U2-OS (■) and the U2-OS/Pt (□) cell lines; (C) JM216 in the U2-OS (▲) and the U2-OS/Pt (Δ) cell lines. Data are means ± S.D. of three duplicate experiments.

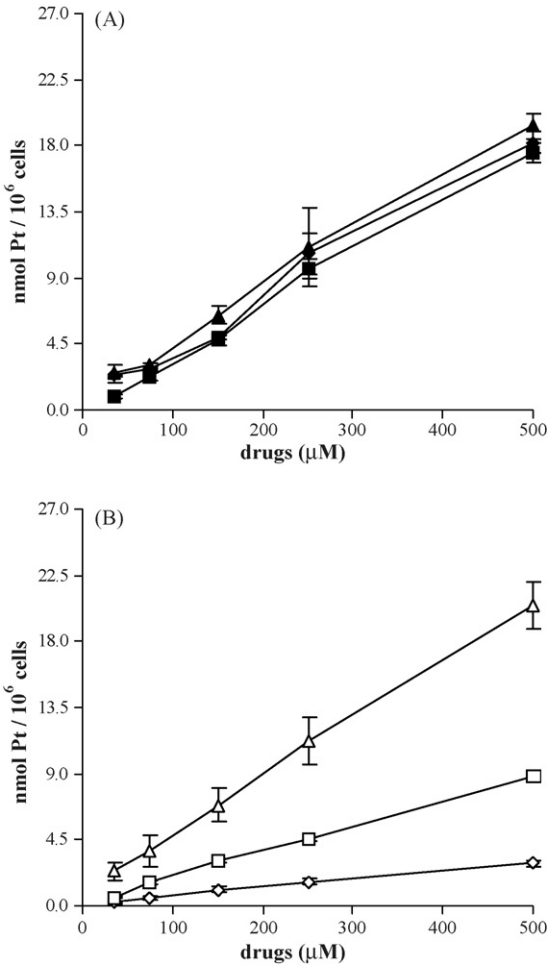

Fig. 2 – Comparison of the levels of Pt accumulation in (A) U2-OS and (B) U2-OS/Pt exposed to CDDP, L-OHP, JM216. Symbols are as reported in Fig. 1.

Figs. 1 and 2. In both cell lines, exposure to all the tested drugs resulted in a concentration-dependent linear increase in Pt levels. For CDDP accumulation, the slope was significantly steeper in U2-OS cells supporting that CDDP uptake was about five-fold higher (mean value) in U2-OS cells than in the resistant variant [slope = 0.036 ± 0.003 (mean ± S.E. of estimate), $r = 0.992$ in U2-OS and slope = 0.006 ± 0.0001, $r = 0.996$ in U2-OS/Pt; $p < 0.0001$, Fig. 1A]. Also the pattern of accumulation of L-OHP was different between parental and resistant cell lines (slope = 0.036 ± 0.001, $r = 0.998$ in U2-OS and slope = 0.017 ± 0.001, $r = 0.999$ in U2-OS/Pt; $p < 0.0001$, Fig. 1B). The accumulation of L-OHP in the sensitive cell line was quite similar to that of CDDP (compare slopes: 0.036 ± 0.001 for L-OHP versus 0.036 ± 0.003 for CDDP, Fig. 2A) while in the resistant variant the accumulation levels were about 2.2-fold higher than CDDP (compare slopes: 0.017 ± 0.001 for L-OHP versus 0.006 ± 0.0001 for CDDP, Fig. 2B). No differences in Pt accumulation levels between sensitive and resistant cell lines were found for JM216 (slope = 0.038 ± 0.002, $r = 0.997$ in U2-OS and slope = 0.039 ± 0.001, $r = 0.999$ in U2-OS/Pt; $p = 0.381$, not significant, Fig. 1C). Summarising these results, it appears that similar levels of Pt were accumulated in U2-OS exposed to the three drugs (Fig. 2A) (respective slopes for CDDP, L-OHP and JM216: 0.036, 0.036 and 0.038) whereas in U2-OS/Pt L-OHP and JM216 accumulated, respectively, about 2.2- and 5.4-fold higher than CDDP (Fig. 2B) (respective slopes for CDDP, L-OHP and JM216: 0.006, 0.017 and 0.039).

3.3. *Drug efflux studies*

Based on the Pt accumulation data, efflux studies were performed in U2-OS and U2-OS/Pt cells exposed to CDDP. The time course of Pt efflux into drug-free medium was examined in U2-OS and U2-OS/Pt cell lines, following a 60 min loading period, with 75 or 400 μM CDDP, respectively (Fig. 3). The retained intracellular Pt levels after incubation in drug-free medium for different times did not show significant differences in efflux (slope = −0.260 ± 0.080, $r = -0.916$ in U2-OS and slope = −0.280 ± 0.123, $r = -0.850$ in U2-OS/Pt; $p = 0.898$, not significant).

3.4. *Relationship between Pt accumulation and drug hydrophobicity*

To assess whether any relationship exists between the hydrophobicity of the drugs and cellular accumulation of Pt in U2-OS and U2-OS/Pt cells, the mean values of the different Pt accumulations in the same range of drug concentrations (35–500 μM) were plotted as a function of ranking the compounds in terms of hydrophobicity (JM216, log $P = -0.16 \pm$ ± 0.16; L-OHP, log $P = -1.65 \pm 0.21$; CDDP, log $P = -2.53 \pm 0.28$) [5]. We found a linear correlation between hydrophobicity and Pt accumulation (using mean data) in both cell lines (Fig. 4). The slope for U2-OS/Pt cells was significantly steeper than that for U2-OS cells (slope = −0.358 ± 0.486, $r = -0.593$ and −3.249 ± 0.201, $r = -0.998$, in U2-OS and U2-OS/Pt cells, respectively, $p < 0.05$, see Fig. 4).

3.5. *Analysis of Pt–DNA adducts*

The amount of Pt bound to DNA after 1 h exposure to various concentrations from 35 to 500 μM of CDDP, L-OHP or JM216 linearly increased in both U2-OS and U2-OS/Pt cells (Figs. 5 and 6). After CDDP treatment, DNA platination levels were 2.3-fold higher in U2-OS cells than in the resistant variant throughout

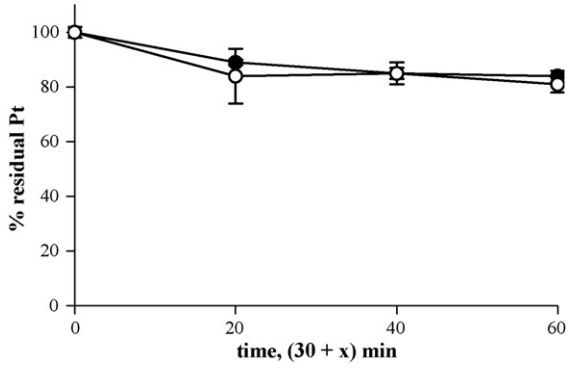

Fig. 3 – Efflux of CDDP over 60 min period from U2-OS (●, 75 μM) and U2-OS/Pt (○, 400 μM) cells. Data are means ± S.D. of three duplicate experiments.

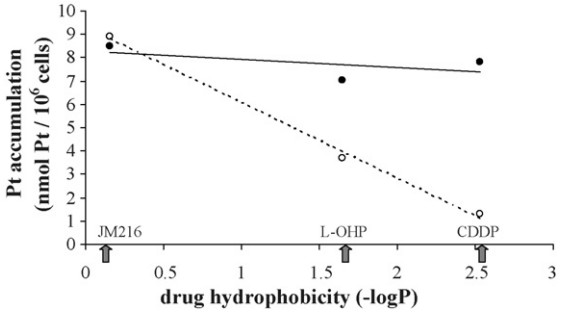

Fig. 4 – Relationship between drug accumulation and hydrophobicity (−log *P*) of the drugs in U2-OS (● and full line) [y = 8.294 − 0.358x; r = −0.593, r^2 = 0.3514] and U2-OS/Pt (○ and dotted line) [y = 9.336 − 3.249x; r = −0.998, r^2 = 0.9962].

the entire range of tested concentrations (compare slopes: slope = 0.0006 ± 0.0001, $r = 0.999$ in U2-OS, and slope = 0.0003 ± 0.0001, $r = 0.999$, in U2-OS/Pt; $p < 0.0001$, Fig. 5A). Also after L-OHP exposure, DNA platination was about two-fold higher in U2-OS than in U2-OS/Pt cells (compare slopes: slope = 0.0005 ± 0.0001, $r = 0.999$ in U2-OS, and slope = 0.0001 ± 0.00001, $r = 0.998$, in U2-OS/Pt; $p < 0.0001$, Fig. 5B). No significant differences in DNA platination were observed between the two cell lines when JM216 was used (slope = 0.0007 ± 0.0001, $r = 0.969$ in U2-OS and slope = 0.0006 ± 0.0001, $r = 0.991$ in U2-OS/Pt; $p = 0.361$, not significant, Fig. 5C). In the parental line, JM216 and CDDP showed similar levels of DNA platination while Pt bound to DNA after L-OHP exposure was slightly lower than the two other drugs (Fig. 6A). In the resistant counterpart JM216 showed quite the same ability to form adducts than in the parental line (Fig. 6B) while DNA platination levels after CDDP and L-OHP exposure were similar between these two drugs and two-fold lower than that of JM216 (Fig. 6B).

4. Discussion

In general, the non-specific diffusion through the double lipid layer of a cell membrane is enhanced and/or fastened when a drug is more liposoluble [5,18,19]. In the U2-OS cell line we found that the most (JM216) and the least (CDDP) hydrophobic drug accumulated at comparable levels (Fig. 1). This behaviour strongly suggests that a facilitated diffusion may be involved in Pt drug accumulation in U2-OS cells. Conversely, in the CDDP-resistant cell line U2-OS/Pt, Pt accumulation becomes a function of drug hydrophobicity, and the most hydrophobic one is privileged (Fig. 2A and B). Furthermore, no significant differences in efflux were observed between sensitive and resistant cell lines, and the R.F. (five-fold for CDDP, two-fold for L-OHP) parallels the reduction of drug accumulation. These findings suggest that the reduced drug accumulation plays an important role in the setting up of the CDDP resistance, and alterations in the cell membrane can be responsible for the reduced drug uptake. These changes, at the selection of the CDDP-resistant variant, seem to cancel the facilitated diffusion

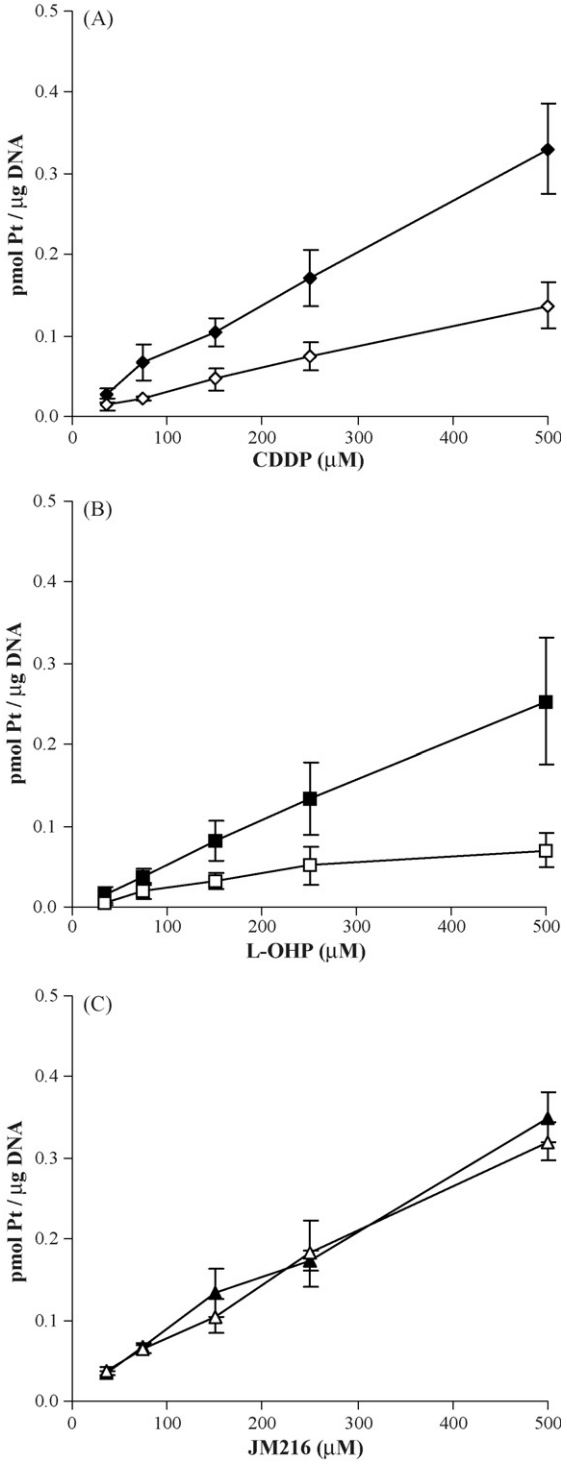

Fig. 5 – Pt–DNA binding after 1 h exposure to increasing concentrations of (A) CDDP in the U2-OS (◆) and the U2-OS/Pt (◇) cell lines; (B) L-OHP in the U2-OS (■) and the U2-OS/Pt (□) cell lines; (C) JM216 in the U2-OS (▲) and the U2-OS/Pt (Δ) cell lines. Data are means ± S.D. of three duplicate experiments.

which appears in the parental cell line and the Pt drug accumulation becomes dependent on the hydrophobicity of the drug. The growing of the resistance to CDDP seems to be overcome by the increasing of hydrophobicity of the Pt

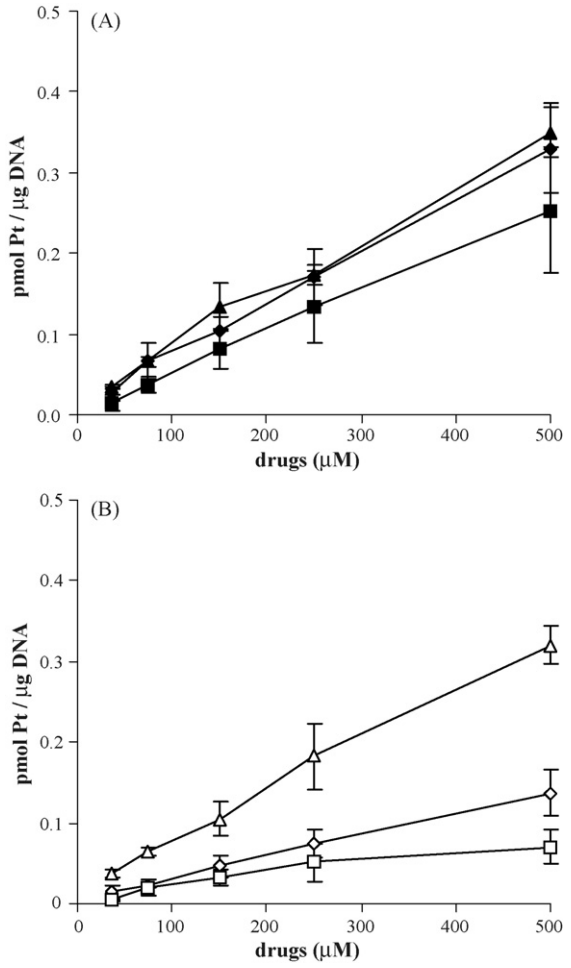

Fig. 6 – Comparison of the levels of Pt–DNA adducts in (A) U2-OS and (B) U2-OS/Pt exposed to CDDP, L-OHP, JM216. Symbols are as reported in Fig. 5.

agent. In line with these conclusions, Beretta et al. [14], considering the same cell lines we used, have found no involvement of CTR1 Cu transporter in the CDDP trafficking in resistant cells, although conflicting data have been reported by other authors in different cell lines [20]. According to our conclusions, Beretta et al. [14] suggest a greater membrane permeability for sensitive cells in comparison with the CDDP-resistant variant. Recently, a significant difference in the physical state of membrane lipids between the sensitive human lung adenocarcinoma A549 and the resistant A549/cisPt cells has been described [21]. Furthermore, the application of surface-enhanced infrared absorption spectroscopy has shown a disordering in the lipid chain packing of resistant cells after application of CDDP [22]. As we attempted to do with A431 cell line [4] we considered the analysis of the cell membrane lipids by proton magnetic resonance (^{1}H NMR) spectroscopy in the resistant and sensitive U2-OS cell lines hoping to obtain indications on the alterations in the mobile lipids and phosphatidylcholine [23,24]. The results still point out alterations of these molecules but we were not able to confer statistical significance to these data, probably because these cell lines are not apt to this kind of measure since they seem to undergo rapid alterations during the ^{1}H NMR measurements.

A further result of our study is that, in the model system A431–A431/Pt, where the R.F. is 2.6, both L-OHP and JM216 were able to circumvent the resistance [4], while in the U2-OS–U2-OS/Pt model, where the R.F. is 5, only the most hydrophobic JM216 overcomes the CDDP-resistance. It is interesting to note that an almost linear relationship seems to relate R.F. and drug hydrophobicity either in the model cell line A431/Pt or in U2-OS/Pt (Fig. 7) but it is intriguing to note that also the pair 41M–41M/cisR4 (R.F. = 4.7) [25], that presents molecular determinants of resistance similar to those of the cell lines we studied, shows a correlation of R.F. versus drug hydrophobicity, similar to that of U2-OS–U2-OS/Pt or A431–A431/Pt. This correlation allows to predict that JM216 could circumvent the resistance. To our knowledge, this result has not been reported in the pair 41M–41M/cisR4 but this is what really happens in the related 41M–41M/cis6 [26]. The relationship between Pt accumulation and hydrophobicity of the drug, and between R.F. and hydrophobicity, that characterize different cell lines, seem to indicate that the change in plasma membrane at the

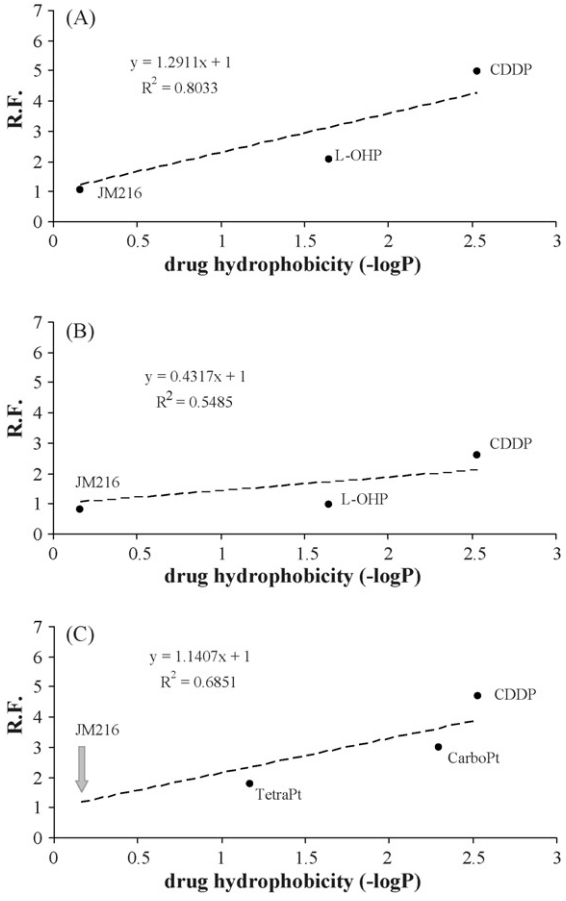

Fig. 7 – Relationship between R.F. and drug hydrophobicity (−log *P*) in (A) U2-OS cells, (B) A431 cells and (C) 41M cells. Assuming that R.F. = 1 is the condition for the overcoming of cell resistance, the fitting of the data was forced through this value. The arrow indicates the hypothetical R.F. value of JM216. −log *P* values are from Screnci et al. [5]; R.F. of graph C are from Kelland et al. [25].

setting up of CDDP-resistance, can be a common and precise event at least in the cell lines with low R.F. This event that could be a small contribution when high levels of resistance are present [3] dictates the amounts of drug accumulation and modulates only the uptake of the lesser lypophilic drugs. Gately and Howell [27] postulated that more lypophilic drugs should not exhibit decreased uptake in resistant cells and now our results seem to support the statement. It must be noted that our results have been obtained using uncharged drugs. Previously we observed that substantially different mechanism(s) of cellular Pt drug accumulation could operate between positively charged and neutral species [28]. Similar conclusions have been suggested to rationalize the transport of the cationic multinuclear Pt complex BBR3464 [13].

Finally, the DNA platination in U2-OS cells is in the order JM216 = CDDP > L-OHP while in U2-OS/Pt cells the sequence is JM216 > CDDP > L-OHP. As for the pairs A431–A431/Pt [4] still it appears that where the accumulation levels of Pt drugs and the rate of reactions with thiol containing species [29] are comparable so allowing the best comparison, L-OHP presents lower DNA platination than the other drugs. The presence of bulky chelated DACH ligand [30–32] could interfere with the rate of DNA platination making the L-OHP cytotoxicity more time-dependent and, consequently, lesser comparable with that of the other drugs.

As far as the cytotoxicity of CDDP and JM216 is concerned despite of their comparable levels of Pt–DNA adducts in U2-OS cell line, the activity of the former is five-fold higher than that of the latter drug. Since the DNA lesions generated by these drugs are repaired *in vitro* with similar kinetics by the mammalian nucleotide excision repair pathway [33] and the mismatch repair complex demonstrates greater preference for CDDP adducts than for JM216 [7], it seems clear that the sensitivity to the tested drugs does not appear to correlate with Pt–DNA levels.

In considering the apoptotic function of p53, current information shows that this function is downregulated in cells expressing the mutant gene but not in cells expressing a wild-type p53 which favours an increased sensitivity to CDDP [7,9]. We noted that this statement is fulfilled by CDDP which is 2-fold more active in U2-OS than in A431 cell line, even though in this latter cell line the Pt–DNA amount is 2.9-fold higher. The wild-type p53 cell feature that exalts the cytotoxicity results also in a greater CDDP-resistance in comparison to a p53 mutant type (R.F. = 5 in U2-OS/Pt, R.F. = 2.6 in A431/Pt).

It is interesting to note that JM216, whose aquated biotransformed product(s) differs from the CDDP analogous only for one ammine substituted by one cyclohexylamine, appears unable to trigger a marked cytotoxicity in a model which retains a wild-type p53. As a matter of fact JM216 presents similar cytotoxicity in U2-OS and in A431 cells. On the basis of these findings it is tempting to speculate that the process leading to drug-induced cell death may differ for different Pt drugs, and the chemical differences in the carrier ligand can trigger important consequences in mediating induction/activation of p53. In this context, also mechanisms of intracellular distribution may play a role in cytotoxic activity of Pt complexes; for example, the involvement of Golgi apparatus or other cellular organelles, such as lysosomes and mithocondria, has been reported in modulating drug effect [34].

While these considerations deserve further studies, it seems clear that in the considered cell lines the hydrophobicity of the drug is important to bypass the CDDP-resistance but this physico-chemical parameter does not affect the pathways supporting drug activity, this possibly due to the biotransformation(s) of the drugs.

In conclusion, in the cell model U2-OS–U2-OS/Pt, the intracellular drug accumulation is an important determinant in CDDP-resistance. In the sensitive cell line, our results are consistent with a Pt uptake sustained by a facilitated diffusion which appears to be lost during the selection of drug-resistant variant(s). The hydrophobicity of the drug seems to be the key to bypass CDDP-resistance; an enhanced hydrophobicity of the drug is required at the growing of the R.F. Furthermore the comparison between U2-OS and A431 cell lines shows that the cytotoxicity of CDDP correlates with the p53 status being greater in U2-OS (wild-type p53) than in A431 (mutant p53). Not only, the p53 wild-type status affords in a greater CDDP-resistance than the p53 mutant type. Surprisingly the p53 status does not seem to confer advantage to the cytotoxicity of JM216 in both cell lines.

Conflict of interest

None declared.

REFERENCES

[1] Kelland LR. Preclinical perspectives in platinum resistance. Drugs 2000;59(Suppl 4):18.
[2] Siddik ZH, Mims B, Lozano G, Thai G. Independent pathways of p53 induction by cisplatin and X-rays in a cisplatin-resistant ovarian tumor cell line. Cancer Res 1998;58:698–703.
[3] Trimmer EE, Essigmann JM. Cisplatin. Essays Biochem 1999;34:191–211.
[4] Martelli L, Di Mario F, Ragazzi E, Apostoli P, Leone R, Perego P, et al. Different accumulation of cisplatin, oxaliplatin and JM216 in sensitive and cisplatin-resistant human cervical tumour cells. Biochem Pharmacol 2006;72:693–700.
[5] Screnci D, McKeage MJ, Galettis P, Hambley TW, Palmer BD, Baguley BC. Relationships between hydrophobicity, reactivity, accumulation and peripheral nerve toxicity of a series of platinum drugs. Br J Cancer 2000;82:966–72.
[6] Lanzi C, Perego P, Supino R, Romanelli S, Pensa T, Carenini N, et al. Decreased drug accumulation and increased tolerance to DNA damage in tumor cells with a low level of cisplatin resistance. Biochem Pharmacol 1998;55:1247–54.
[7] Siddik ZH. Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 2003;22:7265–79.
[8] Houldsworth J, Xiao H, Murty VV, Chen W, Ray B, Reuter E, et al. Human male germ cell tumor resistance to cisplatin is linked to TP53 gene mutation. Oncogene 1998;16:2345–9.
[9] Manic S, Gatti L, Carenini N, Fumagalli G, Zunino F, Perego P. Mechanisms controlling sensitivity to platinum complexes: role of p53 and DNA mismatch repair. Curr Cancer Drug Targets 2003;3:21–9.
[10] Gatti L, Supino R, Perego P, Pavesi R, Caserini C, Carenini N, et al. Apoptosis and growth arrest induced by platinum compounds in U2-OS cells reflect a specific DNA damage recognition associated with a different p53-mediated response. Cell Death Differ 2002;9:1352–9.

[11] Fokkema E, Groen HJ, Helder MN, de Vries EG, Meijer C. JM216-, JM118-, and cisplatin-induced cytotoxicity in relation to platinum–DNA adduct formation, glutathione levels and p53 status in human tumour cell lines with different sensitivities to cisplatin. Biochem Pharmacol 2002;63:1989–96.
[12] Perego P, Righetti SC, Supino R, Delia D, Caserini C, Carenini N, et al. Role of apoptosis and apoptosis-related proteins in the cisplatin-resistant phenotype of human tumor cell lines. Apoptosis 1997;2:540–8.
[13] Perego P, Caserini C, Gatti L, Carenini N, Romanelli S, Supino S, et al. A novel trinuclear platinum complex overcomes cisplatin resistance in an osteosarcoma cell system. Mol Pharmacol 1999;55:528–34 [Erratum in: Mol Pharmacol 1999;55:1108].
[14] Beretta GL, Gatti L, Tinelli S, Corna E, Colangelo D, Zunino F, et al. Cellular pharmacology of cisplatin in relation to the expression of human copper transporter CTR1 in different pairs of cisplatin-sensitive and -resistant cells. Biochem Pharmacol 2004;68:283–91.
[15] Bonetti A, Apostoli P, Zaninelli M, Pavanel F, Colombatti M, Cetto GL, et al. Inductively coupled plasma mass spectroscopy quantitation of platinum–DNA adducts in peripheral blood leukocytes of patients receiving cisplatin- or carboplatin-based chemotherapy. Clin Cancer Res 1996;2:1829–35.
[16] Kirby KS, Cook EA. Isolation of deoxyribonucleic acid from mammalian tissues. Biochem J 1967;104:254–7.
[17] Sambrook J, Fritsch EF, Maniatis T. Molecular cloning: a laboratory manual, 2nd ed., New York: Cold Spring Harbor Lab Press; 1989. p. E.3:E.5.
[18] Moeller N, Kangarloo BS, Puscasu I, Mock C, Krebs B, Wolff JEA. Rational design of platinum chemotherapeutic drugs: hydrophobicity increases cytotoxicity. Anticancer Res 2000;20:4435–40.
[19] Han I, Nguyen T, Yang L, Khokhar AR, Perez-Soler R. Cellular accumulation and DNA damage induced by liposomal *cis*-bis-neodecanoato-*trans*-*R*,*R*-1,2-diaminocyclohexaneplatinum(II) in LoVo and LoVo/PDD cells. Anticancer Drugs 1994;5:64–8.
[20] Samimi G, Howell SB. Modulation of the cellular pharmacology of JM118, the major metabolite of satraplatin, by copper influx and efflux transportes. Cancer Chemother Pharmacol 2006;57:781–8.
[21] Liang X, Huang Y. Physical state changes of membrane lipids in human lung adenocarcinoma A549 cells and their resistance to cisplatin. Int J Biochem Cell Biol 2002;34:1248–55.
[22] Checkhun VF, Solyanik GI, Kulik GI, Tryndiak VP, Todor IN, Dovbeshko GI, et al. The SEIRA spectroscopy data of nucleic acids and phospholipids from sensitive and drug resistant rat tumors. J Exp Clin Cancer Res 2002;21:599–607.
[23] Santini MT, Romano R, Rainaldi G, Filippini P, Bravo E, Porcu L, et al. The relationship between ^{1}H NMR mobile lipid intensity and cholesterol in two human tumor multidrug resistant cell lines (MCF-7 and LoVo). Biochim Biophys Acta 2001;1531:111–31.
[24] Mannechez A, Collet B, Payen L, Lecureur V, Fardel O, Le Moyec L, et al. Differentiation of the P-gp and MRP1 multidrug resistance systems by mobile lipid ^{1}H NMR spectroscopy and phosphatidylserine externalization. Anticancer Res 2001;21:3915–9.
[25] Kelland LR, Mistry P, Abel G, Loh SY, O'Neill CF, Murrer BA, et al. Mechanism-related circumvention of acquired *cis*-diamminedichloroplatinum (II) resistance using two pairs of human ovarian carcinoma cell lines by ammine/amine platinum (IV) dicarboxylates. Cancer Res 1992;52:3857–64.
[26] Loh SY, Mistry P, Kelland LR, Abel G, Harrap KR. Reduced drug accumulation as a major mechanism of acquired resistance to cisplatin in a human ovarian carcinoma cell line: circumvention studies using novel platinum(II) and (IV) ammine/amine complexes. Br J Cancer 1992;66:1109–15.
[27] Gately DP, Howell SB. Cellular accumulation of the anticancer agent cisplatin: a review. Br J Cancer 1993;57:1171–6.
[28] Cere V, Grossi L, Munari D, Pollicino S, Martelli L, Martelli M. Platinum (II) complexes with stereochemically-defined thiepane dioxide diamine ligands as anticancer drugs. Anticancer Res 2006;26:1815–9.
[29] Goodisman J, Hagrman D, Tacka KA, Souid AK. Analysis of cytotoxicities of platinum compounds. Cancer Chemother Pharmacol 2006;57:257–67.
[30] Mishima M, Samimi G, Kondo A, Lin X, Howell SB. The cellular pharmacology of oxaliplatin resistance. Eur J Cancer 2002;38:1405–12.
[31] Woynarowski JM, Chapman WG, Napier C, Herzig MCS, Juniewicz P. Sequence and region-specificity of oxaliplatin adducts in naked and cellular DNA. Mol Pharmacol 1998;54:770–7.
[32] Arnould S, Hennebelle I, Canal P, Bugat R, Guichard S. Cellular determinants of oxaliplatin sensitivity in colon cancer cell lines. Eur J Cancer 2003;39:112–9.
[33] Reardon JT, Vaisman A, Chaney SG, Sancar A. Efficient nucleotide excision repair of cisplatin, oxaliplatin, and bis-aceto-ammine-dichloro-cyclohexylamine-platinum(IV) (JM216) platinum intrastrand DNA diadducts. Cancer Res 1999;59:3968–71.
[34] Kalayda GV, Zhang G, Abraham T, Tanke HJ, Reedijk J. Application of fluorescence microscopy for investigation of cellular distribution of dinuclear platinum anticancer drugs. J Med Chem 2005;48:5191–202.