Cancer Chemother Pharmacol (1995) 35: 371–376
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ORIGINAL ARTICLE

Maurizio Viale · Sergio Cafaggi · Brunella Parodi
Mauro Esposito

Cytotoxicity and cellular accumulation of a new *cis*-diammineplatinum (II) complex containing procaine in murine L1210 cells sensitive and resistant to *cis*-diamminedichloroplatinum (II)

Received: 11 March 1994 / Accepted: 15 July 1994

Abstract The emergence of drug resistance during tumor chemotherapy is one of the main problems associated with cancer treatment, particularly with cisplatin (cis-DDP). In the hope of overcoming this problem, various cis-DDP-derived compounds have been synthesized, and their pharmacological activity was compared with that of cis-DDP. In this paper we report on studies on the cytotoxic activity induced by *cis*-diamminechloro-[2-(diethylamino)ethyl-4-aminobenzoate, N^4]-chlorideplatinum(II) monohydrochloride monohydrate (DPR), a new complex of platinum containing procaine. All experiments were carried out on murine leukemic cells, which were either sensitive (L1210) or resistant (L1210/DDP) to cis-DDP. A tetrazolium dye (MTT) assay conducted 5 days after a 2-h exposure of cells to both drugs was utilized to determine the resistance factor (RF) of L1210/DDP cells as compared with the sensitive wild-type cells. Drug accumulation and efflux, together with the amount of platinum bound to DNA, were also investigated. The activity of DPR on sensitive cells was not significantly different from that of cis-DDP. Conversely, DPR was 4.3 times more effective than cis-DDP on resistant cells. A decreased drug accumulation is one of the mechanisms of resistance to cis-DDP of L1210/DDP cells. However, DPR accumulation was not significantly different in sensitive and resistant L1210 cells. Under culture conditions that yielded similar intracellular platinum concentrations, treatment with DPR produced significantly greater DNA platination than did treatment with cis-DDP in both cell lines. No difference in efflux was observed between L1210 and L1210/DDP cells exposed to either cis-DDP or DPR. Our results show that in parental cells, DPR is as potent as cis-DDP on a molar basis, and it is also minimally cross-resistant with cis-DDP in L1210/DDP cells. A direct implication of our results is that DPR could be useful in those human tumors showing a mechanism of resistance similar to that of L1210/DDP cells.

Key words: Cisplatin-procaine complex · Cisplatin resistance · Cytotoxic activity

M. Viale · M. Esposito (✉)
Istituto Nazionale per la Ricerca sul Cancro, Laboratorio Farmacologia Tossicologica, Viale Benedetto XV, 10, I-16132 Genova, Italy

S. Cafaggi · B. Parodi
Istituto di Analisi e Tecnologie Farmaceutiche, University of Genova, Genova, Italy

Introduction

Cisplatin (cis-DDP) is one of the most active and useful chemotherapeutic agents used alone or in combination for the treatment of various neoplasms, particularly ovarian and testicular carcinomas. In ovarian carcinoma, cis-DDP induces a 50%–65% rate of response in previously untreated patients [29], whereas in patients with advanced testicular carcinoma a cure rate of about 80% has been observed following the administration of cis-DDP-based combination regimens [12]. However, the emergence of drug resistance, a fairly common feature of cis-DDP therapy [25], is one of the main problems associated with the failure of treatment. In this context, multifactorial mechanisms have been considered to explain the acquired resistance of tumor cells. Reduced drug accumulation and drug retention, enhanced inactivation of the drug by thiol compounds, and differential DNA damage have been cited as possible causes of cis-DDP resistance [8, 24].

In an attempt to reduce the toxicity of cis-DDP and to overcome the complication of drug resistance, many analogues of cis-DDP have been developed, some of which have been shown to possess a low degree of toxicity, a good antitumor effectiveness, a broad spectrum of activity, and a low degree of cross-resistance to cis-DDP [1, 3, 17, 18].

DPR is a new platinum(II) complex in which the aromatic amino group of procaine is involved in the coordination with platinum [4]. This new molecule has shown good solubility and stability in water, together with significant in vitro cytotoxicity and in vivo antitumor activity. In this paper we report data concerning the activity of DPR in comparison with cis-DDP on L1210 cells both sensitive and resistant to cis-DDP.

Materials and methods

Chemicals

Cis-DDP was purchased from Sigma (St. Louis, Mo., USA). DPR was synthesized as previously described [4], and its structure is shown in Fig. 1. Platinum agents were dissolved immediately before use in 0.9% saline (for cis-DDP) or water (for DPR) at the opportune concentrations. DPR is highly stable in water [4].

Leukemic cell lines

The murine leukemia cell line L1210 and the cis-DDP-resistant subline L1210/DDP (a kind gift of Dr. F. Zunino, Istituto Nazionale Tumori, Milano, Italy; initially selected at the NCI, Bethesda, Md., USA) were maintained in culture in RPMI 1640 medium containing glutamine (2 mmol/l), gentamycin (100 μg/ml), nonessential amino acids (2%, v/v), 10% fetal calf serum, and 0.003% 2-mercaptoethanol (complete medium). For the resistant L1210/DDP cells, cis-DDP was added to the culture medium at the final concentration of 5 μ*M*. Under these culture conditions, L1210 and L1210/DDP cells had a doubling time of 12.7 and 13.5 h, respectively. To ensure that cells for experimental use were drug-free, resistant cells were cultured without cis-DDP for 4–6 days before experiments.

Chemosensitivity assay

Cells at a density of 10^6/ml were exposed to equimolar concentrations of cis-DDP and DPR for 2 h (final concentration range, 1–256 μ*M*) at 37°C. After 2 h of exposure, cells were washed twice with complete medium, counted, and seeded at 20 cells/well in round-bottomed 96-well microtiter plates in a final volume of 200 μl of complete medium. The low number of cells plated per well was chosen to avoid overgrowth of controls. After 5 days an aliquot of 30 μl of tetrazolium dye (MTT; Sigma, St. Louis, Mo, USA) solution (2 mg/ml in PBS) was added to each well and the plates were incubated for 4 h at 37°C. At the end of the culture period, plates were centrifuged at 275 *g* for 5 min. Then, culture medium was carefully aspirated and 150 μl of 100% dimethylsulfoxide was added.

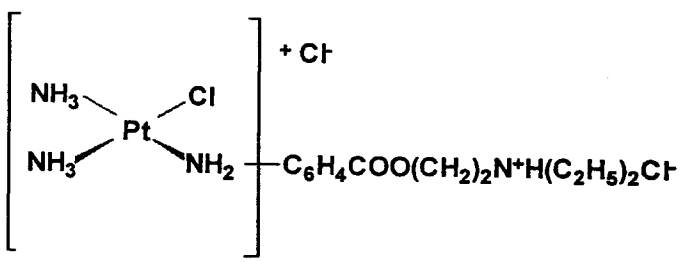

Fig. 1 Scheme representing the chemical structure of DPR

Complete and homogeneous solubilization of formazan crystals was achieved after 10 min of incubation and vigorous shaking of well contents with a multichannel pipette. The absorbance was measured on a microculture plate reader (400 ATC; SLT Labinstruments, Austria) at 540 nm [13]. A 5-day period of culture was chosen because it has been demonstrated that L1210 cells undergo growth arrest in the G2 phase of the cell cycle and that there is a definite loss of viability only after 4 days of incubation with high cis-DDP concentrations [27]. The resistance factors (RFs) were calculated from the ratio between the 50% growth-inhibitory concentrations (IC_{50} values) recorded for L1210/DDP and L1210 cells, respectively.

Measurement of cell volume

Owing to the differences in size between wild-type and resistant cells, we normalized the platinum content by referring it to the cell volume, arbitrarily considering cells as fluid-filled sacs. This was done on the basis of cell diameters calculated by an Epics Elite cytofluorimeter (Coulter Electronics, Hialeah, Fla., USA).

Measurement of cellular drug accumulation

Cell lines were treated with cis-DDP and DPR at equimolar concentrations of 0, 8, 16, 32, and 64 μ*M* for 30, 60, and 120 min at 37°C. Cells were exposed to drugs at a density of 4.5×10^6 cells/ml and were maintained in suspension by continuous agitation for the whole period of drug exposure. Under these culture conditions, no significant loss of viability was observed after exposure of both cell lines to cis-DDP or DPR (0–2.8% loss of viability as determined by the trypan blue dye–exclusion assay). Cells were then collected by centrifugation and rapidly washed twice with cold normal saline to remove extracellular drug. Collected cells were then treated with 1 ml of HNO_3 (65%) at 120°C. The mixture was digested until the HNO_3 evaporated, and the digested cells were dissolved with 10 m*M* HNO_3 (0.2 ml). Platinum concentrations were determined by flameless atomic absorption spectroscopy (AAS; Atomic Absorption Spectrophotometer, Hitachi Model Z-9000 simultaneous spectrophotometer, W. Pabisch Instrument, Milano, Italy) using drying conditions as described elsewhere [4]. Under these conditions the detection limit was 30–50 ng of platinum/ml, and the rate of platinum recovery was about 84%. Intracellular platinum levels were expressed times 10^{-6} ng Pt/μm^3 cellular volume.

Measurement of cellular drug efflux

For drug efflux, cis-DDP was used at a concentration of 32 μ*M* in all cell lines. On the basis of specific drug uptake after 2 h of exposure, DPR was used at a concentration that gave the same platinum uptake per cubic micrometer as 32 μ*M* cis-DDP (24 and 12 μ*M* for L1210 and L1210/DDP cells, respectively). Cells were exposed to drugs at 4.5×10^6 cells/ml for 2 h. After incubation, cells were washed twice with cold normal saline and resuspended in complete medium at a concentration of 2×10^6 cells/ml. Cells were rapidly washed with cold normal saline and harvested at the following time points: 0, 30, 60, 120, and 240 min. Collected cells were then treated as described above. The total cellular efflux of the two drugs was compared by assigning the value of 100% to the drug level achieved after 2 h of exposure and assigning all other values in relation to the 100% value.

Determination of platinum binding to DNA

L1210 cells sensitive and resistant to cis-DDP were exposed to cis-DDP or DPR for 2 h and then washed twice with cold normal

saline. High-molecular-weight DNA was then isolated by means of a DNA-extraction kit (Oncor Inc., Gaithersburg, Md., USA) [9, 26], which utilizes two serial steps of lysis of the cellular and nuclear membranes followed by isolation of DNA by repeated extraction with phenol and chloroform. The DNA content was measured by absorbance at 260 nm (A260). The mean A260/A280 ratio was 2.16 ± 0.29 (SD). DNA was then digested in HNO_3 at 120°C (see above), and the amount of platinum bound to DNA was determined by AAS as previously described and was expressed in picograms of Pt per microgram of DNA.

Statistical analysis

The significance of differences found between cis-DDP and DPR was assessed according to the Mann–Whitney test.

Results

Chemosensitivity assay

The RF of L1210/DDP treated with cis-DDP was 17.7 as evaluated by the MTT assay. On exposure to DPR, the RF shown by L1210/DDP cells was noticeably lower than that observed for cis-DDP, that is, 2.9 (Table 1). Although DPR was 1.4 times less active than cis-DDP on sensitive cells, the difference was not statistically significant. It is noteworthy that in resistant cells our compound was 4.3 times more cytotoxic than cis-DDP (Table 1).

Determination of cell volume

Since the cell volume of resistant cells was slightly different from that of their sensitive counterparts, cellular platinum accumulation was normalized on the basis of cell volume and evaluated as nanograms of Pt per cubic micrometer so as to obtain a more accurate comparison between the drug accumulation of sensitive cells and that of resistant cells. The mean diameters (± SD) of L1210 and L1210/DDP cells were 14.5 ± 1.9 ($n = 5$) and 16 ± 1.6 μm ($n = 6$), respectively. Consequently, the volume of L1210/DDP cells was on average 1.34 times higher than that of L1210 cells.

Table 1. Chemosensitivity of parental and cis-DDP-resistant L1210 cells to DPR and cis-DDP. Data express the mean values for groups of 5–8 data; standard errors are shown in parentheses (*NS*, Not significant)

	MTT assay, IC_{50} (μ*M*)			
	L1210	L1210/DDP	*P* value[a]	RF
cis-DDP	2.3 (0.3)	40.6 (2.5)	< 0.001	17.1
DPR	3.2 (0.6)	9.4 (0.8)	= 0.001	2.9
P value[b]	NS	0.001		

[a] *P* values refer to the comparison between L1210 and L1210/DDP cells exposed to the same Pt compound
[b] *P* values refer to the comparison between cis-DDP and DPR on the same cell line

Assessment of total cellular accumulation of platinum compounds

Platinum accumulation was assessed by AAS after incubation of cells with cis-DDP or DPR for 30, 60, and 120 min. The relationship between the drug concentrations used and the cellular drug accumulation of L1210 and L1210/DDP cells was linear for both cis-DDP and DPR at concentrations of up to 64 μ*M* and for each exposure period considered. When L1210/DDP cells were incubated with cis-DDP, they accumulated a significantly lower amount of platinum than did L1210 cells at any drug concentration used ($P < 0.05$; Fig. 2). Conversely, no significant difference in DPR accumulation was seen between L1210 and L1210/DDP cells ($P > 0.10$). With regard to each cell line tested, DPR accumulated significantly better than cis-DDP in L1210/DDP cells (3 times better on average; *P*-value range, 0.014–0.05), whereas no statistically significant difference in platinum accumulation was observed between L1210 cells exposed to DPR versus cis-DDP (Fig. 2).

Measurement of cellular drug efflux

Drug efflux was evaluated after a 2 h of incubation of cells with drugs. On the basis of uptake data at obtained 2 h, we used a concentration of DPR that induced the same platinum uptake per cubic micrometer as 32 μ*M* cis-DDP. For L1210 and L1210/DDP cells these concentrations were 24 and 12 μ*M*, respectively. Cells were then harvested at 0, 30, 60, 120, and 240 min after drug removal.

No great difference in drug efflux was observed between L1210 and L1210/DDP cells incubated with cis-DDP (Fig. 3). Indeed, after 4 h of incubation, 36.7% and 45.9% of the platinum was removed from L1210 and L1210/DDP cells, respectively. Similarly, no difference in drug efflux was found between L1210 and L1210/DDP cells after incubation with DPR; at 4 h after incubation, 51.5% and 57.9% of the platinum was removed from L1210 and L1210/DDP cells, respectively (Fig. 3).

Determination of platinum binding to DNA

Since decreased accumulation of cis-DDP has been reported in cis-DDP-resistant L1210 cells [24] and because the primary lesion responsible for the cytotoxicity of cis-DDP is probably a result of the

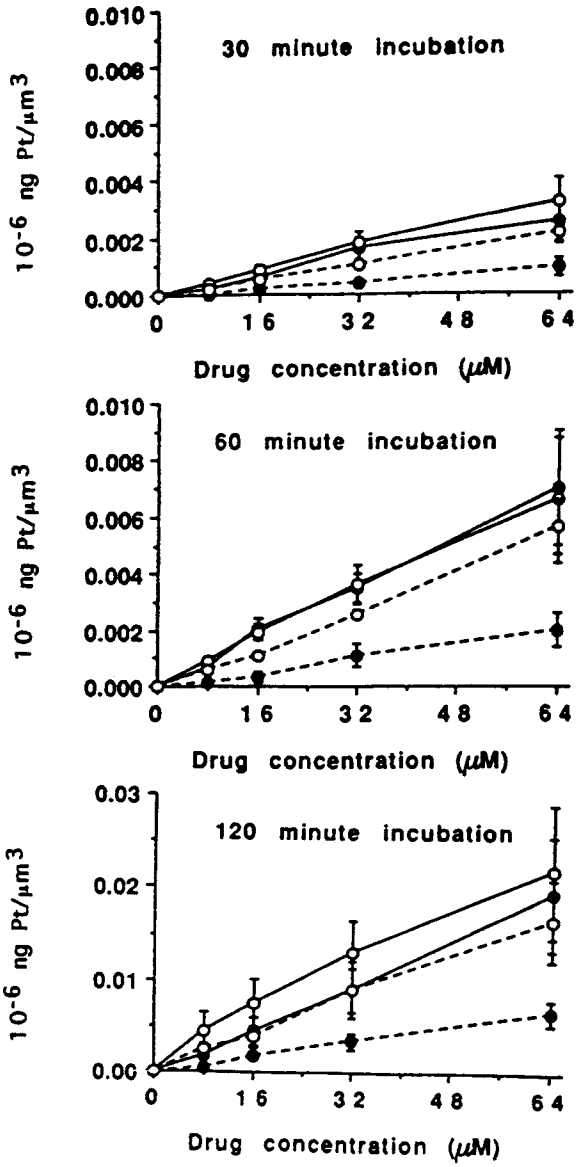

Fig. 2 Relationship between drug concentrations and Pt uptake/μm^3 after 30, 60, and 120 min of incubation with cis-DDP (●) or DPR (○) in L1210 (——) or L1210/DDP (----) cells. Each point represents the means value ± SE for 3 or 4 determinations

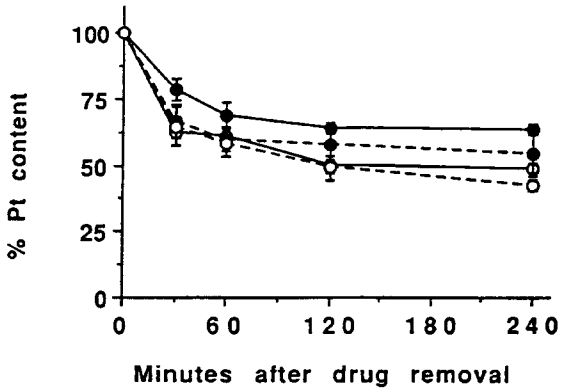

Fig. 3 Relationship between the percentage of Pt content of L1210 (——) and L1210/DDP (----) cells and the time after cis-DDP (●) or DPR (○) removal. Each point represents the mean value ± SE for at least 3 experiments

Table 2 Platinum binding to DNA after exposure of cells to 32 μ*M* DPR or cis-DDP (*NS*, Not significant)

	32 μ*M* cis-DDP		32 μ*M* DPR	*P* value
L1210	23.5 ± 2.6[a]	Ratio = 3.6	75.8 ± 14.4	0.005
L1210/DDP	14.2 ± 0.3	Ratio = 5.2	73.5 ± 15.5	0.028
P value	0.028		NS	

[a] Each value is the mean ± SE for 3–6 experiments and is expressed in pg Pt/μg DNA

Table 3. Platinum binding to DNA after exposure of cells to concentrations of cis-DDP and DPR giving the same Pt uptake/μm^3 as 32 μ*M* cis-DDP in L1210 cells[a] (*NS*, Not significant)

	cis-DDP (32 μ*M*):	DPR (24 μ*M*):	*P* value
L1210	23.5 ± 2.6[b]	46.9 ± 3.4 Ratio = 2	0.028
	cis-DDP (89.8 μ*M*)	DPR (35.6 μ*M*):	
L1210/DDP	34.4 ± 7.3	99.2 ± 11.1 Ratio = 2.9	0.014
P value	NS	0.028	

[a] The Pt uptake at 32 μ*M* cis-DDP observed in L1210 cells was 8.97×10^{-9} ng Pt/μm^3. The concentrations of drugs giving the same Pt uptake/μm^3 as 32μ*M* cis-DDP in L1210 cells are reported in parentheses

[b] Each value is the mean ± SE for 3–6 experiments and is expressed in pg Pt/μg DNA

interaction of cis-DDP with DNA [5, 7, 21], we studied platinum binding to DNA after exposure of sensitive and resistant cells to equimolar concentrations (32 μ*M*) of cis-DDP and DPR.

As expected, the DNA of L1210/DDP cells bound a significantly lower amount of cis-DDP-derived platinum than did the DNA of L1210 cells. Conversely, no difference in the platination of DNA was found between sensitive and resistant cells after exposure to DPR (Table 2). It is also noteworthy that in both cell lines the amount of platinum bound to DNA after DPR exposure was significantly greater than that found after cis-DDP treatment, being 3.6 and 5.2 times greater in L1210 and L1210/DDP cells, respectively (Table 2).

Since the higher accumulation of DPR-derived platinum at the DNA level could depend on differences in cellular platinum uptake between cis-DDP and DPR, we assessed platinum binding to DNA using cis-DDP

and DPR concentrations inducing the same platinum uptake as 32 μM cis-DDP in L1210 cells (i.e., about 8.97×10^{-9} ng Pt/μm^3). Under these conditions, the DNA of L1210 cells bound twice as much DPR-derived platinum as cis-DDP-derived platinum. Similarly, when we considered the DNA of L1210/DDP cells, the amount of platinum linked to DNA was 2.9 times higher in DPR-treated cells than in cis-DDP-treated cells (Table 3).

Discussion

In a previous paper, we reported on the properties of a cationic platinum complex containing procaine as a ligand. As compared with cis-DDP, this complex showed similar or even stronger cytotoxic activity on leukemic cells and a lower degree of in vivo nephrotoxicity [4]. The ^{195}Pt chemical-shift value obtained for DPR was comparable with those presented by Hollis et al. [10] for a series of Pt-triamine complexes, thus suggesting that the ligand has good donor strength despite the low basicity of the aromatic amino group of procaine. Moreover, we showed that procaine could be regarded as a ligand of moderate (normal saline, fetal calf serum) or quite low lability (water, phosphate buffer solution), suggesting that tumor cells are exposed to intact DPR and that DPR has a pharmacological activity of its own [4]. Many derivatives of cis-DDP with antineoplastic activity have been synthesized, and some also seem to be active against mouse leukemias resistant to cis-DDP [14, 28]. Differences in the structure of the carrier ligand and/or leaving groups could be related to the properties of platinum complexes with respect to resistance and accumulation in cis-DDP-resistant murine leukemic cells [14–16, 19, 20].

As is known, multifactorial processes may play a role in resistance to cis-DDP in cancer cell lines [24]; such resistance might result as a combined function of decreased intracellular drug accumulation [2, 22], differential DNA damage [8, 23], and enhanced inactivation by intracellular detoxication systems [6, 24]. In our system no statistically significant difference in activity was observed between cis-DDP and DPR in L1210 cells (Table 1). Conversely, DPR was 4.3 times more cytotoxic than cis-DDP in resistant L1210/DDP cells. An increase in Pt-DNA binding was observed in sensitive and resistant cells exposed to 32 μM DPR. Moreover, after exposure to drug concentrations that induced the same Pt uptake per cubic micrometer as 32 μM cis-DDP in L1210 cells (i.e., the cellular condition under which it is possible to evaluate the intrinsic ability of a compound to target a specific intracellular component), both sensitive and resistant cells showed higher amounts of Pt-DNA adducts after exposure to DPR than after exposure to cis-DDP, thus suggesting that DPR has a greater intrinsic ability to bind DNA than does cis-DDP. This behavior could be related to the structure of the carrier ligand. Finally, no significant difference in efflux was observed between L1210 and L1210/DDP cells exposed to either cis-DDP or DPR.

At the same molar concentration of DPR and cis-DDP, 5.2 times more DPR than cis-DDP was seen to bind to DNA in resistant L1210/DDP cells. Unlike cis-DDP, DPR showed no difference either in accumulation or in DNA platination between L1210 and L1210/DDP cells. This different pattern of cellular platinum accumulation seems to suggest that the transport mechanism for DPR may differ from that of cis-DDP and indicates a way of overcoming one of the mechanisms of resistance of L1210/DDP cells. Moreover, the differences observed in DNA platination suggest that the cytotoxic activity of DPR in L1210/DDP cells might be related at least in part to its ability to induce a greater amount of Pt-DNA adducts than cis-DDP can. Interestingly, these results seem to be very similar to those reported for new platinum(IV) ammine/amine complexes, particularly the platinum compound JM 216 [bis-acetatoammine dichloro (cyclohexylamine) platinum(IV)], which is capable of partially circumventing cell resistance as a result of its enhanced accumulation [20]. We have previously shown that tumor cells are exposed to intact DPR and that this compound is transformed into uncharacterized Pt complexes, without any relevant loss of procaine ligand, in phosphate buffer medium at a concentration similar to that found inside the cell [4].

As has been suggested for other cationic Pt complexes that possess only one leaving group [10, 11], DPR should not form bifunctional cross-links with DNA as classic cis-DDP analogues do. Without loss of NH_3 or procaine, such a molecule would produce only monofunctional lesions on DNA. Experiments to investigate this aspect are in progress. Preliminary findings seem to suggest the capability of DPR to form interstrand cross-links on calf-thymus DNA in a dose-dependent manner and to a greater extent than cis-DDP (unpublished data). As has been suggested for the novel platinum(IV) amine/amine complex JM 221 in both sensitive and resistant cell lines and for tetraplatin in one resistant cell line [22], the lack of cross-resistance of DPR in L1210/DDP cells might depend on two factors: the greater intracellular accumulation of DPR as compared with cis-DDP and the inability of L1210/DDP cells either to repair efficiently the high numbers of DNA adducts or to tolerate the high levels of platinum species present after DPR exposure. However, although L1210 and L1210/DDP cells exposed to 32 μM *DPR* showed virtually identical DNA-Pt levels, *L*1210/DDP cells were nonetheless about 3 times more resistant to DPR. Hence, although the high DNA platination observed in DPR-treated L1210/DDP cells may contribute to overcoming the drug resistance, the DPR sensitivity/resistance of this cell line should also

be determined by additional mechanism(s) involving DNA repair and/or intracellular thiol content. Further information about the fine mechanism of action of DPR is needed.

In summary, DPR shows evident activity on resistant L1210/DDP cells. Whereas the fine mechanism(s) of its effectiveness remain unresolved, it is likely that better cellular accumulation and DNA platination are critical. Our results also suggest that DPR might be useful in those human tumors that show a mechanism of resistance to cis-DDP similar to that displayed by the L1210/DDP cell line.

Acknowledgements We thank Dr. G. Melioli and his colloborators (Laboratory of Clinical Pathology, IST, Genova, Italy) for their help in determining cell volumes and Dr. B. Patrick for the revision of the manuscript. This work was partially supported by a grant from Lega Italiana per la Lotta contro i Tumori, Section of Sanremo, Italy.

References

1. Brown DB, Khokhar AR, Hacker MP, Lokys L, Burchenal JH, Newman RA, McCormack JJ, Frost D (1982) Synthesis and antitumor activity of new platinum complexes. J Med Chem 25: 952
2. Bungo M, Fujiwara Y, Kasahara K, Nakagawa K, Ohe Y, Sasaki Y, Irino S, Saijo N (1990) Decreased accumulation as a mechanism of resistance to *cis*-diamminedichloroplatinum(II) in human non-small cell lung cancer cell lines: relation to DNA damage and repair. Cancer Res 50: 2549
3. Bruchenal JH, Kalaher K, Dew K, Lokys L (1979) Rationale for development of platinum analogs. Cancer Treat Rep 63: 1493
4. Cafaggi S, Esposito M, Parodi B, Vannozzi MO, Viale M, Pellecchia C, Fulco RA, Merlo F, Zicca Z, Cadoni A, Bignardi G (1992) Synthesis and antitumor activity of a new *cis*-diammineplatinum(II) complex containing procaine hydrochloride. Anticancer Res 12: 2285
5. Comess KJ, Burstyn JN, Essigmann JM, Lippard SJ (1992) Replication inhibition translesion synthesis on templates containing site-specifically placed *cis*-diamminedichloroplatinum(II) DNA adducts. Biochemistry 31: 3975
6. De Graeff A, Slebas RJC, Rodenhuis S (1988) Resistance to cisplatin and analogues: mechanisms and potential clinical implications. Cancer Chemother Pharmacol 22: 325
7. Eastman A (1987) The formation, isolation and characterization of DNA adducts produced by anticancer platinum complexes. Pharmacol Ther 34: 155
8. Eastman A, Schulte N (1988) Enhanced DNA repair as a mechanism of resistance to *cis*-diamminedichloroplatinum(II). Biochemistry 27: 4730
9. Gross-Bellard M, Oudet P (1973) Isolation of high-molecular-weight DNA from mammalian cells. Eur J Biochem 36: 32
10. Hollis LS, Amundsen AR, Stern EW (1989) Chemical and biological properties of a new series of *cis*-diammineplatinum(II) antitumor agents containing three nitrogen donors: *cis*-$[Pt(NH_3)_2(N\text{-donor})C]^+$. J Med Chem 32: 128
11. Hollis LS, Sundquist WI, Burstyn JN, Heiger-Bernays WJ, Bellon SF, Ahmed KJ, Amudsen AR, Stern EW, Lippard SJ (1991) Mechanistic studies of a novel class of trisubstituted platinum(II) antitumor agents. Cancer Res 51: 1866
12. Horwich A, Easton D, Husband J, Nicholas D, Peckham MJ (1987) Prognosis following chemotherapy for metastatic malignant teratoma. Br J Urol 59: 578
13. Hussain RF, Nouri AME, Oliver RTD (1993) A new approach for measurement of cytotoxicity using colorimetric assay. J Immunol Methods 160: 89
14. Kamisango K, Matsumoto T, Akamatsu K, Morikawa K, Tashiro T, Koizumi K (1992) Antitumor activity and cellular accumulation of a new platinum complex, (−)-(R)-2-aminomethylpyrrolidine(1,1-cyclobutanedicarboxylato)platinum(II) monohydrate, in cisplatin-sensitive and -resistant murine P388 leukemia cells. Jpn J Cancer Res 83: 304
15. Kelland LR, Murrer BA, Abel G, Giandomenico CM, Mistry P, Harrap KR (1992) Ammine/amine platinum(IV) dicarboxylates: a novel class of platinum complex exhibiting selective cytotoxicity to intrinsically cisplatin-resistant human ovarian carcinoma cell lines. Cancer Res 52: 822
16. Kraker AJ, Moore CW (1988) Accumulation of *cis*-diamminedichloroplatinum(II) and platinum analogues by platinum-resistant murine leukemia cells in vitro. Cancer Res 48: 9
17. Lee FH, Canetta R, Issell BF, Lenaz L (1983) New Platinum complexes in clinical trials. Cancer Treat Rev 10: 39
18. Lelieveld P, Van der Vijgh WJF, Veldhuizen RW, Van Velzen D, Van Putten LN, Atassi G, Dangy A (1984) Preclinical studies on toxicity, antitumor activity and pharmacokinetics of cisplatin and three recently developed derivatives. Eur J Cancer Clin Oncol 20: 1087
19. Loh SY, Mistry P, Kelland LR, Abel G, Harrap KR (1992) 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 66: 1109
20. Mellish KJ, Kelland LR, Harrap KR (1993) In vitro platinum drug chemosenstivity of human cervical squamous cell carcinoma cell lines with intrinsic and acquired resistance to cisplatin. Br J Cancer 68: 240
21. Micetich K, Zwelling LA, Kohn KW (1983) Quenching of DNA: platinum(II) monoadducts as a possible mechanism of resistance to *cis*-diamminedichloroplatinum(II) in L1210 cells. Cancer Res 43: 3609
22. Mistry P, Kelland LR, Loh SY, Abel G, Murrer BA, Harrap KR (1992) Comparison of cellular accumulation and cytotoxicity of cisplatin with that of tetraplatin and amminedibutyratodichloro(cyclohexylamine)-platinum(IV) (JM 221) in human overian carcinoma cells lines. Cancer Res 52: 6188
23. Parker RJ, Eastman A, Bostik-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
24. Richon VM, Schulte N, Eastman A (1987) Multiple mechanisms of resistance to *cis*-diamminedichloroplatinum(II) in murine leukemia L1210 cells. Cancer Res 47: 2056
25. Scanlon KJ, Kashani-Sabet M. Miyachi H, Sowers LC, Rossi J (1989) Molecular basis of cisplatin resistance in human carcinomas: model systems and patients. Anticancer Res 9: 1301
26. Smith LJ, Brylan RC, Edmundson KB, Nutkis JE, Wakeland EK (1987) In vitro transformation of human B-cell follicular lymphoma cells by Epstein-Barr virus. Cancer Res 47: 2062
27. Sorenson CM, Eastman A (1988) Mechanism of *cis*-diamminedichloroplatinum(II)-induced cytotoxicity: role of G2 arrest and DNA double-stand breaks. Cancer Res 48: 4484
28. Wilkoff LJ, Dulmadge EA, Trader MW, Harrison SD Jr, Griswold DP Jr (1987) Evaluation of *trans*-tetrachloro-1,2-diamminecyclohexaneplatinum(IV) in murine leukemia L1210 resistant and sensitive to *cis*-diamminedichloroplatinum(II). Cancer Chemother Pharmacol 20: 96
29. Wiltshaw E, Evans B, Rustin B, Gilbey E, Baker J, Baker GA (1986) A prospective randomized trial comparing high-dose cisplatin with low dose cisplatin and chlorambucil in advanced ovarian carcinoma. J Clin Oncol 4: 722