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Original Paper

Cisplatin Combined with the New Cisplatin–Procaine Complex DPR: *In Vitro* and *In Vivo* Studies

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The administration of combinations of platinum compounds is considered as a useful alternative therapeutic strategy to avoid the complications of toxic events during cancer chemotherapy in order to obtain a therapeutic advantage. On the basis of previous *in vitro* and *in vivo* findings, suggesting an antitumour activity of the new cisplatin-derived compound *cis*-diamminechloro-[2-(diethylamino)ethyl 4-amino-benzoate, N^4]-chlorideplatinum(II) monohydrochloride monohydrate (DPR), we investigated the effectiveness of the combination of cisplatin (DDP) and DPR *in vitro* on murine leukaemic cells, which were either sensitive (P388) or resistant (L1210/DDP) to DDP, and on the murine M5076 reticulum cell sarcoma, and *in vivo* in BDF1 female mice transplanted with P388 leukaemic cells or cisplatin-resistant L1210/DDP leukaemic cells. The contemporaneous exposure *in vitro* to both platinum compounds gave a significantly higher cell growth inhibition than that expected on the basis of dose–response curves for single agents in all tumour models tested. *In vivo*, the combinations of DDP plus DPR elicited significant enhancement over the activity of the drugs alone both in the ascitic and solid P388 models. The combined treatment of 10 mg/kg DDP and 14 mg/kg DPR yielded 62.5% tumour-free mice compared with 6.2% with 10 mg/kg DDP alone, the best single agent. It is noteworthy that the combined application of DDP and DPR was also very effective in the solid cisplatin-resistant L1210/DDP model, inducing a significant reduction in the volume of tumour. A therapeutic advantage was achieved with combination treatments that had no effect on platinum-mediated body weight loss and were generally well tolerated by the mice. At equitoxic concentrations of DPR and carboplatin, the treatment with DDP plus DPR proved to have a higher efficacy against this tumour model compared to that observed after the combined treatment with DDP and carboplatin. In summary, the combination of DDP and DPR showed a therapeutic advantage over single drug treatment and has demonstrated promise at the preclinical level in its ability to circumvent acquired resistance to DDP both *in vitro* and *in vivo*. Copyright © 1996 Elsevier Science Ltd

Key words: cisplatin, cisplatin–procaine complex, combined action *in vitro* and *in vivo*

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INTRODUCTION

DPR (*cis*-diamminedichloroplatinum (II)) is a new platinum triamine complex where the aromatic amino group of procaine is involved in coordination with platinum. This new molecule has shown *in vitro* cytotoxic activity against human and murine leukaemic cells [1]. In particular, DPR was able to overcome the *in vitro* resistance to DDP (*cis*-diammine-chloro-[2-(diethylamine) ethyl 4-amino-benzoate, N^4]-chlorideplatinum (II) monohydrochloride monohydrate) in L1210/DDP murine leukaemic cells [2], and this activity seemed to be linked, at least in part, to the ability of DPR to bind DNA to a greater extent than DDP. *In vivo* DPR,

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tested in BDF1 mice bearing P388 leukaemia, gave maximal antitumour activity comparable to that obtained with the maximal tolerated dose of DDP, but without its dose-limiting nephrotoxicity [1]. It is also of note that DPR has a much greater solubility (>50 mg/kg) and stability in water than DDP [1]. On the basis of these findings, DPR seems to be a promising platinum anticancer agent. Nevertheless, there is no information on its precise mechanism of action, although its interaction with cellular DNA seems to be critical as it is for cisplatin.

It has been suggested that administration of platinum analogues given in combination may be an alternative dose-escalation strategy and that their *in vitro* interaction could be synergistic, additive or even antagonistic, depending on the cell line involved [3, 4]. Therefore, in order to study in more depth the platinum compound, we examined the feasibility and the effectiveness of the association of DDP and DPR. We report herein the results of experimental antitumour studies on the effects of this combination treatment.

MATERIALS AND METHODS

Chemicals

DPR was synthesised as previously described [1]. For animal studies, the antitumour effect of drugs given in combination was investigated at doses of DDP (Sigma Chemical Co., St Louis, Missouri, U.S.A.) and DPR lower than their 10% lethal dose (LD_{10}).

Cell line

Murine P388 and L1210/DDP (kindly provided by Dr F. Zunino, Istituto Nazionale Tumori, Milano, Italy, initially selected at NCI, Bethesda, Maryland, U.S.A.) leukaemic cells were maintained in exponential growth in RPMI 1640 medium containing glutamine (2 mmol/l), gentamycin (100 μg/ml), 0.003% 2-mercaptoethanol and 10% fetal calf serum (complete medium). L1210/DDP cells were maintained in complete medium containing 5 μM DDP. Murine M5076 (kindly provided by Dr. S. Filippeschi, Istituto Mario Negri, Milano, Italy) were maintained in RPMI 1640 medium containing glutamine (2 mmol/l), gentamycin (100 μg/ml), 1 mM sodium pyruvate and 15% heat-inactivated donor horse serum (complete medium). For *in vivo* studies, before experiments, P388 and L1210/DDP cells were maintained in BDF1 mice by i.p. (intraperitoneal) weekly transplantation of 10^6 cells in 0.1 ml of 0.9% NaCl solution (normal saline).

In vitro *studies*

The combined effect of DDP and DPR was tested *in vitro* using continuous exposure to both compounds for 72 h. Cells were plated in round-bottomed microtitre plates at 400/well (P388) and 800/well (L1210/DDP) or in flat-bottomed microtitre plates at 4000/well (M5076) and treated with DDP dissolved in normal saline (final concentration range: 0.025–0.4 μM), DPR dissolved in distilled water (final concentration range: 0.025–1.04 μM) and selected combinations of the two compounds. In combination experiments for P388 and M5076, we used DDP and DPR concentrations which were lower than their respective mean 50% inhibiting concentration (IC_{50}) (i.e. for P388, DDP concentration range: 0.025–0.1 μM; DPR concentration range: 0.025–0.2 μM; for M5076, DDP concentration range: 0.25–0.06 μM; DPR concentration range: 1.04–0.125 μM). For L1210/DDP cells, we used a combination of their equitoxic concentrations IC_{30} (30% inhibiting concentration) and IC_{50}. The final volume of each well was 200 μl. After 3 days, an aliquot of 30–50 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) solution (2 mg/ml in PBS) was added to each well and 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% dimethylsulphoxide was added. Complete and homogeneous solubilisation of formazan crystals was achieved after 10 min of incubation and the wells vigorously shaken with a multichannel pipette. The absorbance was measured by a microculture plate reader 400 ATC (SLT Labinstruments, Austria) at 540 nm [5]. IC_{50}s were calculated on the basis of probit analysis of single dose–response curves.

In vivo *studies*

DPR was prepared in distilled water to expose mice to doses of 0.5, 6, 7, 10 and 14 mg/kg body weight. DDP was prepared at a concentration 1 mg/ml in normal saline to treat mice with 1, 3, 6 and 10 mg/kg body weight. Female BDF1 mice (18–22 g) were implanted i.p. with 10^5 P388 cells, or s.c. with 10^6 P388 or L1210/DDP cells, in the left flank on day 0. Both DDP and DPR were given i.p. 24 h later (day 1). When both drugs were administered together they were injected from separate syringes into different parts of the peritoneal cavity. DDP was injected immediately prior to DPR. All animals were allowed free access to standard diet (4RF/25 Italiana Mangimi, Settimo Milanese, Italy) and water. Tests were carried out using groups of 4–12 mice for each dose. Control tumour-bearing animals were given i.p. injection of normal saline. For i.p. tumours, mortality was monitored daily for 60 days. The *in vivo* activity of each treatment against P388 leukaemic cells was assessed by determining the median survival time (MST). When mice were implanted s.c., they were sacrificed at day 7 (for L1210/DDP tumour) or 13 (for P388 tumour) in order to avoid unnecessary suffering due to overdeveloped tumour masses. Before sacrificing the animals, perpendicular tumour diameters were measured by a caliper and tumour volumes calculated using the formula [6]:

$$\frac{\text{length} \times (\text{width})^2}{2}.$$

The animals' weights were recorded during the course of the experiments and the percentage change in body weight on day 7 or 13 was used as an index of drug toxicity.

Data analysis

In vitro. The dose–response curves were initially drawn for single compounds by the probit analysis, then the analysis of combination treatments was made by the isobole method [7]. For a combination of compounds A and B, the combination index D is calculated by the equation:

$$\frac{Ac}{Ae} + \frac{Bc}{Be} = D$$

where Ac and Bc are the concentrations of the compounds in the combination and Ae and Be are the concentrations of

platinum compounds which alone gave the same magnitude of effect [8]. If $D < 1$, the effect of the combination was considered synergistic; if $D = 1$, the effect was considered simply additive; finally, if $D > 1$, the effect was considered antagonistic. Each experiment was performed in quadruplicate to allow the calculation of P values using the Mann–Whitney test. Experimental D values for additivity were calculated using combinations of two preparations of DDP or DPR.

In vivo. The survival distribution for each group of mice was estimated with the Kaplan–Meier step function [9]. The Wilcoxon rank sum test of Breslow, modified for censored survival data [10], was used for testing survival differences between groups (at 0.05 level of significance), as allowed by the procedure 1L, BMDP statistical Software [11]. The Mann–Whitney test was used to evaluate the significance of differences in the volume of tumour masses, while the χ^2 test was used for comparing data regarding the percentage of tumour-free mice (TFM).

RESULTS

Chemosensitivity in vitro

The mean $IC_{50} \pm$ S.D. in P388 cells for DDP and DPR were 0.11 ± 0.04 μM and 0.23 ± 0.02 μM, respectively. The analysis of data by the isobole method showed that the activity of the combinations was always significantly higher than that expected on the basis of single dose–response curves, except in the case of the combination of 0.2 μM DPR with 0.025 μM DDP, where the association was simply additive (Table 1). Similar results were obtained when L1210/DDP cells were exposed to DDP and DPR. The IC_{30} and IC_{50} for DDP and DPR were 4.8 and 7 μM, and 0.72 and 1.7 μM, respectively, as obtained by the mean dose–response curve for single agents. When combined together in these experimental conditions, DDP and DPR gave a significant cellular synergism, as shown in Table 2. Table 3 shows the results of similar experiments performed on murine M5076 cells. The mean $IC_{50} \pm$ S.D. for DDP and DPR were 0.48 ± 0.11 μM and 1.99 ± 0.40 μM, respectively. A significant cellular synergism was evident in all combinations but two, where the combined effect of DDP plus DPR was simply additive.

Table 1. In vitro *activity of DDP plus DPR combinations on P388 cells*

DPR concentration (μM)	DDP concentration (μM) 0.1	0.05	0.025
0.2	$D = 0.65$ $P = 0.0007$* Syn	$D = 0.61$ $P = 0.0013$ Syn	$D = 0.94$ $P = 0.305$ Add
0.1	$D = 0.64$ $P = 0.0032$ Syn	$D = 0.56$ $P = 0.0007$ Syn	$D = 0.72$ $P = 0.0132$ Syn
0.05	$D = 0.57$ $P = 0.0032$ Syn	$D = 0.45$ $P = 0.0007$ Syn	$D = 0.60$ $P = 0.0044$ Syn
0.025	$D = 0.60$ $P = 0.0132$ Syn	$D = 0.49$ $P = 0.0007$ Syn	$D = 0.65$ $P = 0.006$ Syn

*P values were calculated according to the Mann–Whitney test by comparing D values for the combinations of DDP plus DPR with the experimental D values for additivity obtained using combinations of two DDP solutions [D value for additivity: 0.99 ± 0.02 (SE)].

Syn, synergy; Add, additivity.

Table 2. In vitro *activity of DDP plus DPR combinations on L1210/DDP* cells*

DPR concentrations	DDP concentrations IC_{30} (4.8 μM)	IC_{50} (7 μM)
IC_{30} (0.72 μM)	$D = 0.88$ $P = 0.10$† Add	$D = 0.79$ $P = 0.05$ Syn
IC_{50} (1.7 μM)	$D = 0.64$ $P = 0.02$ Syn	$D = 0.64$ $P = 0.02$ Syn

*The resistance factor for DDP of L1210/DDP was 17. †P values were calculated according to the Mann–Whitney test by comparing D values for the combinations of DDP plus DPR with the experimental D values for additivity obtained using combinations of two DDP or DPR solutions [mean D value for additivity: 1.01 ± 0.05 (SE)].

Syn, synergy; Add, additivity.

Antitumour activity against P388 i.p. tumour

A single administration of increasing doses of 1, 3 and 6 mg of DDP alone per kg as well as 0.5, 6 and 10 mg of DPR alone per kg resulted in increased survival patterns, suggesting a positive dose–response relationship (Table 4). The highest activities for single agents DDP and DPR were obtained at the dose of 6 mg of DDP per kg and 10 mg of DPR per kg (Table 4).

Combination treatment with the two platinum complexes yielded an apparent synergistic action. Data in Table 4 show that a single i.p. treatment on day 1 with a suboptimal dose of DDP used alone (1 mg/kg) in combination with suboptimal doses of DPR (0.5 or 6 mg per kg body weight) produced %ILS (percentage increase in life span) values significantly greater than those observed after single DDP

Table 3. In vitro *activity of DDP plus DPR combinations on murine M5076 reticulosarcoma cells*

DPR concentration (μM)	DDP concentration (μM) 0.25	0.125	0.06
1.04	$D = 0.69$ $P = 0.002$* Syn	$D = 0.83$ $P = 0.10$ Add	$D = 0.82$ $P = 0.10$ Add
0.52	$D = 0.70$ $P = 0.02$ Syn	$D = 0.80$ $P = 0.05$ Syn	$D = 0.68$ $P = 0.002$ Syn
0.25	$D = 0.70$ $P = 0.002$ Syn	$D = 0.69$ $P = 0.02$ Syn	$D = 0.68$ $P = 0.05$ Syn
0.125	$D = 0.60$ $P = 0.002$ Syn	$D = 0.58$ $P = 0.002$ Syn	$D = 0.59$ $P = 0.02$ Syn

*P values were calculated according to the Mann–Whitney test by comparing D values for the combinations of DDP plus DPR with the experimental D values for additivity obtained using combinations of two DDP or DPR solutions [mean D value for additivity: 0.97 ± 0.04 (SE)].

Syn, synergy; Add, additivity.

Table 4. Effect of combination treatment with DDP and DPR on the survival of BDF1 mice bearing i.p. P388 leukaemic cells

Treatment		Survival distribution*					% change in weight at day 7
		Quartiles (days)		% 60-day			
DDP (mg/kg)	DPR (mg/kg)	MST	75–25%	survivors	*P* value	ILS (%)‖	Mean
		12	12–13	0			+8.8
	0.5	13	13–15	0	NS	8	+8.5
	6	15	15–16	0	<0.01	25	+3.3
	10	17	15–17	0	<0.01	42	+4.5
1		13	12–17	0		8	+2.9
1	0.5	19	19–22	0	<0.01	58	+3.6
1	6	24	23–27	0	<0.01	100	+2.4
1	10	21	19–22	8	<0.01	75	+2.3
3		18	17–21	8		50	+2.1
3	0.5	23	22–24	0	<0.05	92	+2.6
3	6	26	23–40	20	<0.01	117	+2.9
3	10	†	‡	100	<0.01	†	+2.7
6		23	22–27	17		92	−4.4
6	0.5	32	31-§	33	NS	167	−3.3
6	6	†	39-§	67	<0.05	†	−5.2
6	10	†	‡	83	<0.01	†	−4.7

*Kaplan–Meier product-limit method was used to estimate survival curves [10, 11]. Quantiles: MST (median survival time), 75% and 25% are the days at which 50%, 75% and 25% of animals are still alive; †MST is >60 days, ‡proportion of 60-day survivors is >75%; § = proportion of 60-day survivors is >25%; *P* = computed probabilities in testing the significance of differences between survival distributions (generalized Wilcoxon test statistics); NS = not significant. ‖ILS = increase in life span (%) compared to the median survival of tumour-bearing animals given normal saline alone.

treatment ($P < 0.01$). Treatment with increasing doses of DPR (0.5, 6 and 10 mg/kg) together with DDP resulted in increasing proportions of tumour-bearing mice surviving for 60 days compared to those observed in mice receiving DDP alone. Combination treatments with 3 and 6 mg of DDP per kg plus 10 mg of DPR per kg were the most effective (100% and 83% 60-day survivors, respectively) compared to the most effective single DDP treatment (i.e. 6 mg/kg, 17% 60-day survivors; Table 4). The addition of DPR did not cause any notable body weight loss compared to DDP alone. A slight but statistically non-significant decrease in body weight was observed only when DDP was administered at the dose of 6 mg/kg in combination with 6 mg of DPR per kg (Table 4). The findings reported in Table 5 show that, when DDP doses of 3 and 6 mg/kg were administered along with a fixed dose of 14 mg/kg DPR (i.e. a dose amounting to approximately 50% of its LD_{10} dose [1]), the therapeutic efficacy was maintained, as measured by the increase of %ILS and 60-day survivors. Because our previous findings [12] indicated that procaine hydrochloride (P.HCl) improves the therapeutic index of DDP, one experiment was also carried out to investigate whether the enhanced therapeutic response observed in leukaemic mice treated with DDP plus DPR may be related to the decomposition of DPR into its parent forms. Table 6 shows that the optimal therapy achieved by combining 6 mg of DDP per kg plus a dose of DPR amounting to its LD_{10} dose (i.e. 28 mg/kg) is not a consequence of the decomposition of DPR into its parent forms, since treatment with an equimolar combination of DDP and P.HCl caused reduction in %ILS, while the combination DDP plus DPR resulted in an increased survival pattern (Table 6). Table 7 shows data on the effect of carboplatin on the antitumour activity of DDP. The co-administration of DDP and carboplatin gave a %ILS in agreement with that waited for a simple additivity, with 33.3% of survivors at day 60 (Table 7). It is noteworthy that, at equitoxic concentrations of all three platinum drugs, an enhanced therapeutic efficacy of DDP against P388 leukaemia can be achieved by combination with DPR (Table 5) compared to that observed after com-

Table 5. Effect of DDP with or without 14 mg/kg of DPR on survival of mice bearing i.p. P388 leukaemia

	Therapeutic response			
	Experiment 1		Experiment 2	
Treatment*	ILS† (%)	60-day survivors (alive/total)	ILS (%)	60-day survivors (alive/total)
DDP, 3 mg/kg	50	0/6	58	1/6
DDP, 6 mg/kg	83	1/6	108	1/6
DPR, 14 mg/kg	42	0/6	50	0/6
DDP, 3 mg/kg + DPR, 14 mg/kg	> 400	3/6	> 400	6/6
DDP, 6 mg/kg + DPR, 14 mg/kg	> 400	5/6	> 400	4/6

*Drugs were given 24 h later than the inoculum of 10^5 P388 leukaemic cells. DDP and DPR were given i.p. in 0.9% NaCl (DDP) and in water (DPR). †ILS = increase in life span (%) compared to the median survival of tumour-bearing animals given normal saline alone (i.e. MST = 12 days for experiment 1 and 13 days for experiment 2).

Table 6. Effect of DPR (28 mg/kg) and its parent forms (DDP and P.HCl) on the antitumour activity of DDP (6 mg/kg) in BDF1 mice bearing i.p. P388 leukaemic cells

Treatment	MST (days)*	ILS (%)†	60-day survivors (alive/total)	% change in weight at day 7 (mean)
DDP + DPR	>60	>400	5/6	−7.1
DDP + DDP‡ + P.HCl§	10	−17	2/6	−28

*MST = median survival time. †ILS = increase in life span (%) compared to the median survival of tumour-bearing animals given normal saline alone (MST = 12 days). ‡The dose of DDP equivalent to that of 28 mg/kg DPR was 14.2 mg/kg body weight. §The dose of P.HCl equivalent to that of 28 mg/kg DPR was 12.9 mg/kg body weight.

Table 7. Effect of combination treatment of DDP and carboplatin on the survival of BDF1 mice bearing i.p. P388 leukaemic cells

Treatment*				
DDP (mg/kg/i.p.)	Carboplatin (mg/kg/i.p.)	MST	ILS† (%)	60-day survivors (alive/total)
		13	0	0/6
6		23	77	0/6
6	65	26	100	2/6
	65	23	77	0/6

*Drugs were given 24 h later than the inoculum of 10^5 P388 leukaemic cells. Carboplatin was administered in water at its 50% LD_{10} dose immediately after DDP in 0.9% NaCl. †ILS = increase in life span (%) compared to the median survival of tumour-bearing animals given normal saline alone.

bined therapy of DDP plus carboplatin (Table 7) under similar experimental conditions.

Antitumour ativity against P388 and L1210/DDP s.c. tumours

The effect of the combination of 3 and 10 mg/kg DDP simultaneously administered with 14 mg/kg DPR was evaluated on s.c. implanted P388 tumours (Table 8). At the dose used, DPR produced only a slight and non-significant reduction of tumour volume (23%), as compared to untreated controls, while DDP at doses of 3 and 10 mg/kg reduced the tumour volume by 49% and 94%, respectively. When DDP and DPR were simultaneously administered, the reduction in tumour volume was significantly increased (on average by 70%), without a marked decrease in body weight as compared to animals treated with the same doses of DDP given alone. The most intriguing result concerned the difference in the percentage of TFM at day 13 which was

Table 8. Effect of DDP with and without DPR on P388 leukaemia implanted subcutaneously

Treatment						
DDP (mg/kg)	DPR (mg/kg)	n	MTV* (mean ± SD)	P value†	% weight change at day 13	Tumour-free mice (%)‡
		17	2.74 ± 1.16		+23.1	0
	14	18	2.11 ± 1.32	0.10	+20.0	0
3		6	1.40 ± 0.67		+10.9	0
3	14	6	0.52 ± 0.12	0.004	+11.6	0
10		16	0.17 ± 0.18		+6.9	6.2
10	14	8§	0.05 ± 0.09	<0.05	+3.1	62.5‖

*MTV, mean tumour volume (cm^3) determined on day 13. †Groups of mice were compared by the Mann–Whitney test. ‡Mice with no measurable tumour at day 13. §Two mice died before day 13. ‖$P < 0.025$ (χ^2 test) versus 10 mg/kg DDP alone.

Table 9. Effect of DDP with and without DPR on L1210/DDP implanted subcutaneously

Treatment						
DDP (mg/kg)	DPR (mg/kg)	n	MTV* (mean ± SD)	P value†	% weight change at day 7	Tumour-free mice (%)‡
		5	0.45 ± 0.07		+10.8	0
	7	5	0.48 ± 0.13	NS§	+6.0	0
	14	5	0.47 ± 0.13	NS	+10.7	0
6		5	0.50 ± 0.35	NS	+2.9	0
6	7	5	0.45 ± 0.36	NS	+5.0	0
6	14	5	0.33 ± 0.15	NS	+1.8	0
10		5	0.35 ± 0.19	NS	−3.8	0
10	7	5	0.21 ± 0.11	0.016	−3.8	0
10	14	5	0.15 ± 0.09	0.014	−7.0	20

*MTV, mean tumour volume (cm^3) determined on day 7. †Groups of mice were compared with untreated controls by the Mann–Whitney test. ‡Mice with no measurable tumour at day 7. §Compared to untreated controls; NS, not significant.

10 times higher in mice treated with 10 mg of DDP per kg plus DPR than in mice given only DDP at the same dose ($P < 0.025$).

Our data on P388 were confirmed in mice bearing s.c. implanted L1210/DDP tumour. The combination of DDP and DPR, at doses which were not effective in reducing tumour volume when given alone, caused a significant tumour reduction at day 7 post-implantation as compared to controls (Table 9). Both doses of 7 and 14 mg of DPR per kg, when co-administered in association with 10 mg of DDP per kg, significantly reduced the tumour compared to controls. Although a relative tumour reduction was also seen in mice treated with 6 mg of DDP per kg plus 14 mg of DPR per kg, the effect was statistically non-significant (Table 9).

DISCUSSION

These studies show that combination treatments of P388 and L1210/DDP leukaemic cells with DDP and DPR yield an apparent synergistic action both *in vitro* and *in vivo*. Our data show that a therapeutic advantage may be obtained by the combination of low doses of the two platinum compounds.

DPR is a new anticancer compound obtained by the chemical synthesis of DDP and procaine [1]. In previous works [1, 13], DPR showed good *in vitro* cytotoxic and *in vivo* antitumour activity, without the undesired dose-limiting nephrotoxicity of DDP. Moreover, DPR was able to overcome *in vitro* resistance to DDP in L1210/DDP cells, and this seemed to be linked, in part, to a greater ability of DPR to bind DNA and to accumulate in the cells compared with DDP [2].

As previously reported in *in vitro* studies [1], the mechanistic possibility that DPR may influence the activity of DDP after *in vivo* decomposition into its parent forms does not seem likely. It might be argued from these findings that the procaine moiety in the DPR complex plays a crucial role in its mechanisms of action. It is known that platinum agents may interact with cellular DNA and/or other specific molecular determinants of cytotoxicity at different rates [14, 15]. Our previous studies have shown that: (i) the accumulation rate of platinum following exposure of P388 cells to DPR was twice that observed for DDP [1]; (ii) at least part of the non-cross-resistance observed in DDP-resistant L1210/DDP cell line appears to be due to the greater intracellular accumulation and DNA platination of DPR [2]. According to our *in vitro* studies, DPR can form interstrand cross-links on DNA in spite of the presence of only one leaving group (unpublished observation). Although it is accepted that the monofunctional adducts of DDP have no biological activity, there was evidence for efficacy against murine and the human tumour system of some platinum triamine cations [16, 17]. How, then, can the interstrand cross-link formation without DPR decomposition be explained? One possibility is that the DPR complex may function as a carrier, acting as a protected DDP species and enhancing its transport into the cells. If this assumption is correct, then it would be expected that the free DDP from DPR is the true active species within the cells. Therefore, under conditions yielding similar intracellular platinum concentrations, the final adducts on DNA should be the same for DDP and DPR. While this possibility cannot be excluded, it seems inconsistent with the observations that, at the same intracellular platinum concentrations, DPR produced significantly higher platination than DDP, both in sensitive and cisplatin-resistant L1210 cells [2]. Another possibility is that DPR may form unstable monofunctional adducts within DNA which, owing to the low intracellular chloride concentration or DNA conformation (i.e. base sequence), could generate bifunctional adducts from a two-step reaction, such as has been suggested for other monofunctional agents [18].

How interaction of DDP and DPR should be explained is still unclear. It is possible that the parallel induction of DNA damage by DDP and the decrease of the repair of this damage induced by DPR may be another explanation for the enhanced therapeutic effect produced by the combination treatment. Other favourable pharmacological interactions, maybe pharmacokinetics, should also be hypothesised.

Overall, these data suggest that DPR may have a role in multidrug therapy which includes DDP as a component of the treatment. However, further studies on human tumour xenografts are required to evaluate the potential application of this interesting combination of platinum antitumour agents.

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