Preclinical *in vitro* evaluation of hematotoxicity of the cisplatin–procaine complex DPR

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We evaluated *in vitro* the inhibitory effect of *cis*-diamine-chloro-[2-(diethylamino) ethyl 4-amino-benzoate, N^4]-chlorideplatinum(II) monohydrochloride monohydrate (DPR) on colony formation by granulocyte/macrophage (CFU-GM) peripheral blood progenitor cells, representing a method to quantitate the toxicity of drugs to the hematopoietic system, and human leukemic cell lines. The results were compared with those obtained exposing cells to cisplatin and carboplatin. Our data showed that while DPR had a significantly better cytotoxic activity than cisplatin and carboplatin against HL60 and K562, and than carboplatin against Molt 4 cells, it showed 12 and 43 times less inhibitory effect on CFU-GM than cisplatin and carboplatin, respectively. These results suggest that the myelosuppressive activity of DPR could be lower than that of cisplatin and carboplatin, and, furthermore, that leukemic cells represent a preferential target for its cytotoxic activity compared to normal committed hemopoietic progenitor cells. All our results speak in favor of a better therapeutic index for DPR than for the other platinum compounds considered here. *Anti-Cancer Drugs* 14:163–166 © 2003 Lippincott Williams & Wilkins.

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Introduction

The cisplatin–procaine complex *cis*-diaminechloro-[2-(diethylamino) ethyl 4-amino-benzoate, N^4]-chlorideplatinum(II) monohydrochloride monohydrate (DPR) [1] is a highly soluble platinum triamine complex containing the local anesthetic procaine as ligand of moderate or low lability. This compound showed a much higher solubility than cisplatin (>50 versus 2 mg/ml) and a good *in vitro* antiproliferative activity [1–3] characterized by a significant selectivity for neuroblastoma, microcitoma, ovarian and leukemia cell lines (manuscript in preparation). *In vivo* DPR, tested in BDF1 mice bearing P388 leukemia, showed a comparable antitumor activity to cisplatin but without its typical nephrotoxicity [1], while in *in vitro* experimental models DPR confirmed its lower nephrotoxicity [4] and showed a significantly lower neurotoxic effect [5] than its parent compound. Finally, studies *in vitro* and *in vivo* demonstrated that our compound was able to synergize with different anticancer agents usually administered to cancer patients [3,6,7].

In the present short report we have investigated the *in vitro* hematotoxicity of DPR comparing the results of the inhibition of granulocyte/macrophage (CFU-GM) colony formation, obtained from human peripheral blood progenitor cells, with those obtained, in the same experimental conditions, by the inhibition of human leukemic tumor cell lines characterized by different cell compartment origins.

Materials and methods

Drugs

Cisplatin (Sigma, St Louis, MO), carboplatin (Sigma) and DPR [1] were dissolved at the opportune concentrations in normal saline (cisplatin) and distilled water in order to allow the exposure of cells to final scalar concentrations of 10^{-3} to 10^{3} μM. Each drug solution was prepared freshly before use.

Separation of peripheral blood mononuclear cells

Mononuclear non-adherent cells (MNACs) were obtained by standard techniques from peripheral blood of five normal subjects. Blood samples were layered over a lymphocyte separation medium (Cedarlane Laboratories, Hornby, Ontario, Canada) and centrifuged for 30 min at 400 *g*. Light density mononuclear cells were collected from the plasma/medium interface, washed twice and resuspended in RPMI 1640 medium containing 20% fetal calf serum and 2 mmol/l L-glutamine (complete medium). Adherent cells and lymphocytes were removed from mononuclear cells by standard methods.

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CFU-GM assay

MNACs were incubated at a concentration of 5×10^5 cells/ml for 1 h with the various concentrations of drugs (range 10^{-3} to 10^3 μM). They were then washed twice with complete medium. MNACs (1×10^6/ml) were plated in 35-mm Petri dishes, in duplicate, in methylcellulose medium containing 20% fetal calf serum and 10% conditioned medium from human bladder carcinoma cell line 5637, as a source of colony stimulating factor. Dishes were incubated at 37°C, 5% CO_2 and 100% humidity for 13 days. CFU-GM (clusters of at least 50 cells) were counted using an inverted microscope. Results are expressed as the mean of colony growth as percent of controls ± SD.

Leukemia cell assay

HL60, K562 and Molt-4 leukemia cells were incubated in vials with drugs as described. They were then washed twice with complete medium. Aliquots of 333 μl of cell suspensions containing 3×10^2/ml HL60 and K562 cells or 4×10^2/ml Molt-4 cells were then plated in 24-well plates (in triplicate) in a semisolid methylcellulose medium containing 20% fetal calf serum. Cultures were then incubated for 9 days at 37°C with 5% CO_2 and 100% humidity. Colonies were defined as clusters of at least 40 cells. Results are expressed as the mean of colony growth as percent of controls ± SD.

Statistics

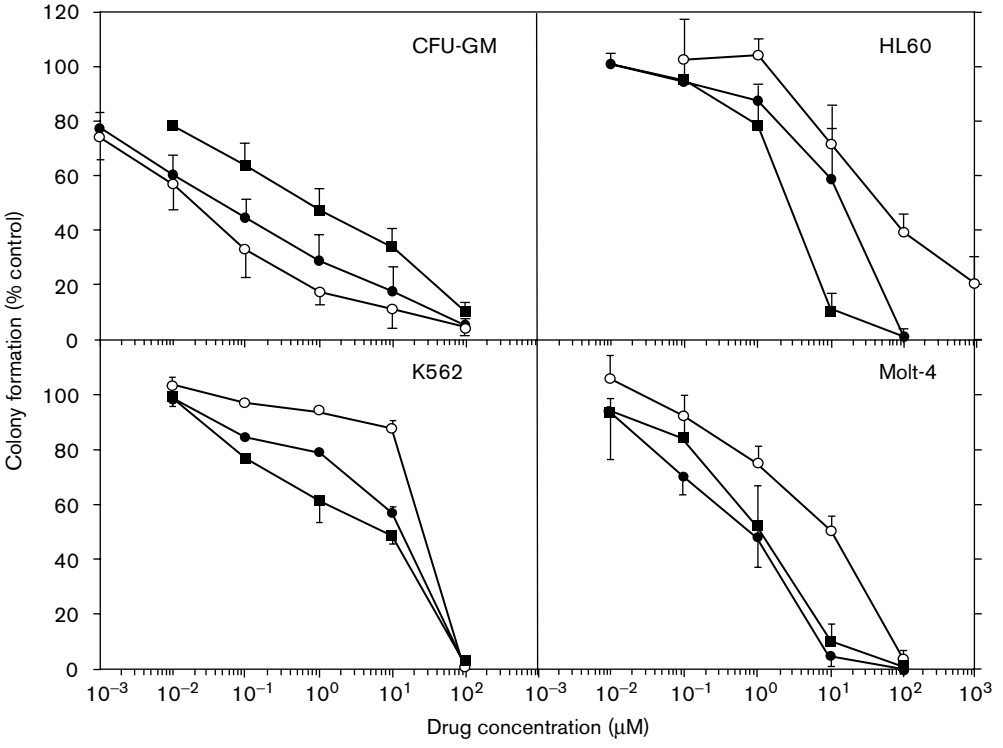
Data were analyzed by Student's *t*-test for unpaired data. The level of significance was set at $p < 0.05$.

Results

Effect of DPR, cisplatin and carboplatin on CFU-GM growth

Colony formation by CFU-GM was inhibited by DPR, cisplatin and carboplatin in a concentration-dependent manner. As hoped, DPR showed the lowest inhibiting potential over the whole range of concentrations used (Fig. 1). As shown in Table 1, this was confirmed in terms of IC_{50}, with a value for DPR which was 12 times higher than that of cisplatin (0.86 ± 0.33 versus 0.07 ± 0.06 μM, $p < 0.01$) and 43 times higher than that of carboplatin (0.02 ± 0.01 μM, $p < 0.01$). Also considering the IC_{90} values, which have been suggested to represent a better predictive parameter for the hematotoxicity of anticancer drugs [8,9], we obtained comparable results with the IC_{90} for DPR (104.6 ± 15.8 μM) about 5 and 9 times higher than those of cisplatin (22.6 ± 16.9 μM, $p < 0.001$) and carboplatin (11.6 ± 8.5 μM, $p < 0.001$), respectively (Table 1).

Fig. 1



Comparison of DPR (solid squares), cisplatin (solid circles) and carboplatin (open circles) inhibiting the potential of colony formation from leukemia cells (HL60, K562 and Molt-4) and peripheral blood CFU-GM precursors.

Table 1 Concentrations of cisplatin, carboplatin and DPR inhibiting 50 and 90% of colony formation from leukemia cells and peripheral blood GM-CFU precursors [evaluation by long-term (9 and 13 days) cultures in a semisolid methylcellulose medium]

Cell model	Cisplatin	Carboplatin	DPR
IC_{50} (μM)			
Molt-4	$0.44 \pm 0.19^{a,*}$	$6.05 \pm 1.14^{**}$	1.20 ± 0.56
HL60	$6.73 \pm 2.54^{***}$	$57.4 \pm 18.40^{**}$	2.17 ± 1.16
K562	$5.10 \pm 1.02^{**}$	$176.61 \pm 30.27^{**}$	2.49 ± 0.44
CFU-GM	$0.07 \pm 0.06^{***}$	$0.02 \pm 0.01^{***}$	0.86 ± 0.38
IC_{90} (μM)			
CFU-GM	$22.55 \pm 16.86^{**}$	$11.63 \pm 8.48^{**}$	104.63 ± 15.80

[a]Values were obtained from the inhibition curves showed in Figure 1. They represent the mean $\pm$ SD of four to six experiments.
$^{*}p<0.05$, $^{**}p<0.001$, $^{***}p<0.01$, as compared to DPR-treated cells and determined by the Student's *t*-test for unpaired data.

Effect of DPR, cisplatin and carboplatin on leukemia cell colony formation

As depicted in Figure 1 and Table 1, DPR was more cytotoxic than cisplatin and carboplatin against the promyelocytic HL60 (2.17 ± 1.16 versus 6.73 ± 2.54 versus $57.40 \pm 18.40\,\mu$M, respectively) and the erythroleukemia K562 (2.49 ± 0.44 versus 5.10 ± 1.02 versus $176.61 \pm 30.27\,\mu$M, respectively) cell lines, and more cytotoxic than carboplatin against the Molt-4 T cell leukemia (1.20 ± 0.56 versus $6.05 \pm 1.14\,\mu$M).

Discussion

In spite of the general experience achieved with its frequent use in oncological diseases, particularly in those of the ovary, testes, and head and neck, antitumor activity of cisplatin is often associated with severe dose-limiting adverse effects, in particular nephrotoxicity, neurotoxicity and ototoxicity [10,11]. In order to synthesize new analogs of this platinum compound, endowed with improved solubility, reduced toxicity and a different selectivity, several years ago we synthesized a monofunctional platinum triamine complex named DPR. This complex was obtained by the synthesis of cisplatin and procaine [1] on the basis of the hypothesis that procaine could improve the therapeutic index of cisplatin [12] through its complexation with cisplatin and the formation of a less toxic, but similarly active compound. DPR revealed good cytotoxic and anticancer activities in different *in vitro* and *in vivo* tumor models [1–3], showing a considerably lower toxicity than cisplatin against different non-neoplastic tissues, as determined in *in vitro* and *in vivo* experimental models [1,4,5]. Furthermore, DPR was less embryotoxic than cisplatin [13,14], as demonstrated in the mouse and chick embryonic experimental models (submitted manuscripts).

As is known, many anticancer drugs can cause dose-limiting myelosuppression. Although nephrotoxicity, neurotoxicity and ototoxicity are considered the main dose-limiting adverse effects of cisplatin, cisplatin-induced myelosuppression has been sometimes reported, particularly with high-dose regimens [15,16]. As opposed to cisplatin, the use of carboplatin in clinical practice is often hampered by the onset of myelosuppression, which typically represents its main dose-limiting side effect [17,18].

On the basis of these suggestions we thought to verify the potential hemotoxicity *in vitro* of DPR, and, for comparison, of the parent compound cisplatin and the more myelotoxic carboplatin, utilizing the method of colony formation by peripheral blood CFU-GM obtained from healthy donors. The *in vitro* cytotoxicity against committed progenitors, evaluated as the inhibition of CFU-GM growth, is a validated test for predicting clinical neutropenia [8,19]. By using of this assay it is possible to forecast the toxicity of a molecule to the hematopoietic system [20].

In our experimental conditions DPR showed a lower inhibiting activity against CFU-GM growth than cisplatin and carboplatin. It is of note that also considering the IC_{90}, which has been suggested to represent a better predictive parameter for the MTD of anticancer drugs [8,9], DPR was much less hematotoxic than the reference compounds.

In general, the efficacy of a drug, particularly an anticancer drug, depends on its therapeutic index, which represents the ratio between its efficacy and toxicity. In order to preclinically estimate the possible therapeutic index of DPR, in comparison to cisplatin and carboplatin and relatively to its myelosuppressive activity, we also tested the sensitivity of some leukemia cell lines against these platinum drugs using the same colony formation assay. The choice of HL60, Molt-4 and K562 leukemia cell lines was mainly due to the fact that they originated from progenitor cells belonging to the hematopoietic system. Moreover, leukemia cells were also shown to be highly sensitive to the DPR inhibiting effect, as demonstrated by the MTT assay and using a disease-oriented strategy [21] (manuscript in preparation).

Our data clearly showed that DPR is more effective against this tumor histotype than the other two platinum compounds and, moreover, that leukemia cells may represent a preferential target for DPR cytotoxicity, compared to normal committed progenitor cells.

Conclusions

The fact that in the CFU-GM predictive test, DPR showed a lower hematotoxicity than cisplatin and, above all, than the more myelosuppressive anticancer drug carboplatin, is a significant indication of its low myelosuppressive action. This observation is in accordance with our previous findings indicating the lower general toxicity

[1,4,5] of DPR, compared to cisplatin. Furthermore, the high antiproliferative activity of DPR against leukemia cells suggests its preferential activity against cell lineages mutated in a leukemic sense and indicates in favor of a better therapeutic index of DPR, compared to the other platinum compounds. Altogether these findings demonstrate that DPR may be a potentially useful antitumor drug, particularly with regard to its very low hematotoxicity.

Acknowledgment

Several years ago Dr Mauro Esposito synthesized DPR on the basis of experiments showing the protective activity of procaine hydrochloride against cisplatin-induced nephrotoxicity. This paper is dedicated to his memory.

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