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Comparative Effect of Cisplatin, Spiroplatin, Carboplatin, Iproplatin and JM40 in a Human Myeloid Clonogenic Assay

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Abstract—*The relative toxicities of cisplatin and its analogs, spiroplatin, carboplatin, iproplatin and JM40, were tested against normal human progenitor myeloid cells (CFU-GM) in a clonogenic assay. Cells obtained from five bone marrows were incubated for* 60 min *with various drug concentrations and plated. The mean inhibitory concentrations for* 50% *of the bone marrow colonies* (IC_{50}) *were* 15.6 μg/ml *for cisplatin,* 0.4 μg/ml *for spiroplatin,* 56.3 μg/ml *for carboplatin,* 36.3 μg/ml *for iproplatin and* 179.5 μg/ml *for JM40. Ratios of the* IC_{50}*s of the analogs with cisplatin as reference drug closely followed the corresponding ratios of the clinical maximum tolerated doses. This correlation between the CFU-GM assay results and the clinical myelotoxicity suggests that the assay is adequate for predicting myelotoxicity* in vitro *and selecting* in vitro *drug concentrations for the human tumor clonogenic assay.*

INTRODUCTION

DIFFERENT experimental approaches, including the clonogenic assay, have been tried to determine the cytotoxicity of chemotherapeutic agents. In the clonogenic assay developed by Pike and Robinson, marrow progenitor cells (CFU-C) differentiate into colonies of mature granulocytes, eosinophils, monocytes and macrophages and can be adequately grown in agar [1]. Widely used to study human hematopoiesis, this assay may be useful for evaluating the relative myelosuppressive effect of cytotoxic drugs.

Cisplatin has proven to be a mainstay of the treatment of several human neoplasms. The major toxic effect limiting its clinical use is nephrotoxicity. However, with proper hydration and diuresis the kidneys can be partially protected so that the dose-limiting toxicity becomes myelosuppression [2].

Another approach to circumventing cisplatin nephrotoxicity has been the development of analogs. Three analogs, carboplatin, iproplatin and JM40, showed in phase I studies minimal or no renal toxicity at high doses and a level of antitumor activity that was equal to or in excess of that of cisplatin and myelosuppression [3–5]. In other trials, spiroplatin, a fourth analog, also produced dose-limiting myelosuppression [6]. The present study was designed to investigate and compare the relative cytotoxic effect of cisplatin and these four analogs on human bone marrow myeloid stem cells in a clonogenic assay and to evaluate its predictive value for the clinic.

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MATERIALS AND METHODS

Marrow samples

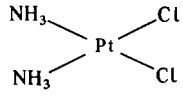
Four milliliters of human bone marrow were taken from the posterior iliac crest of five healthy volunteers. Each sample was anticoagulated with 10 U/ml heparin (Upjohn Company, Kalamazoo, Michigan). Mononuclear cells were separated at room temperature by Ficoll/Hypaque density centrifugation at 800 ***g*** for 20 min. The cells at the interface were collected, washed twice and resuspended in Dulbecco's medium enriched with 20% fetal calf serum (FCS) (Gibco, Paisley, U.K.). Approximately 3×10^6 mononuclear cells per ml of bone marrow were retrieved.

Drugs

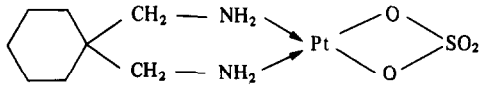
Cisplatin and its four analogs were obtained from Bristol-Myers Co., Wallingford, Connecticut. Drug structures are illustrated in Fig. 1. Each drug was dissolved in calcium and magnesium free Hank's balanced salt solution (HBSS) (Gibco, Paisley, U.K.), to give the appropriate range of concentrations from 10^{-3} to 10^{-6} M for all but spiroplatin which was tested from 10^{-4} to 10^{-7} M. Preliminary experiments defined the concentration inducing a 100% cell kill for each drug.

Drug testing

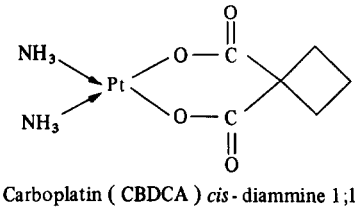
5×10^5 marrow cells were incubated for 60 min at 37°C in a 2 ml final volume of culture medium enriched with 20% fetal calf serum and the various drug concentrations. This was done in a controlled atmosphere containing 7.5% CO_2 and 100% H_2O. The incubations were terminated by adding a 10-fold excess of Dulbecco's medium at 4°C. The cells were then centrifuged at 800 ***g*** for 10 min. The pellets were resuspended in culture medium and plated according to a modified Pike and Robinson technique as previously reported [7].



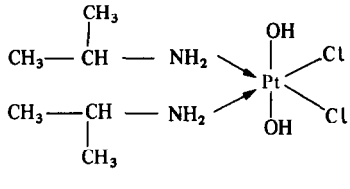
Cisplatin (CDDP) *cis* - diammine - dichloroplatinum II MW 300



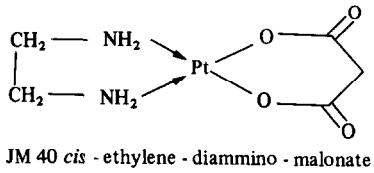
Spiroplatin (TNO - 6) 1,1 - diamminomethylcyclohexane sulfate platinum II MW 419



Carboplatin (CBDCA) *cis* - diammine 1;1 - cyclobutanedicarboxylate platinum II MW 371



Iproplatin (CHIP) *cis* - dichloro - *trans* - dihydroxy - bis - isopropylamine platinum IV MW 418



JM 40 *cis* - ethylene - diammino - malonate - - platinum (II) MW 357

Fig. 1. Structures of the five platinum analogs used in the experiments.

Culture system

Human placental conditioned medium (HPCM) was added to the soft agar for stimulating colony growth. Cells were resuspended in 3.5 ml of culture medium enriched with 20% FCS, 10% HPCM and 0.28% agar. One milliliter of the suspension was aliquoted into 35 mm plastic Petri dishes and incubated for 7 days at 37°C with a 7% CO_2 humidified atmosphere. Colonies, defined as groups of more than 40 cells, were counted with an Olympus inverted microscope at 40× magnification. The experiments were set up in triplicate with the necessary controls.

Data analysis

A minimum of 30 colonies per control plate was required for evaluation. Colony counts of the three plates for a particular drug concentration were averaged to obtain one data point. The drug concentrations inhibiting growth by 50% were calculated as inverse regression estimates with linear regression on the logarithmic concentration for each series of experiments. For this calculation, nonlinear points were eliminated. For those cases in which only two concentrations remained after nonlinear points were eliminated, the inverse regression was still done, realizing that no estimate of variability could be obtained and linearity could no longer be checked. It was felt that the effect of any potential bias would be minor, particularly on the reported means. This affected only cisplatin and carboplatin for the human progenitor myeloid cells. The geometric means and standard errors were then calculated based on a log-normal distribution [8].

RESULTS

The mean number of control CFU-C colonies in the triplicates ranged from 108 to 324 with a median value of 195. Sensitivity to a given drug varied among the five normal bone marrows. The IC_{50} (drug concentration capable of inhibiting 50% growth) for each drug is listed in Table 1. Cisplatin induced 50% cell kill at an average dose of 5.2×10^{-5} M, an IC_{50} concentration similar to iproplatin. Only one analog, spiroplatin, proved more toxic. Carboplatin reached the same level of inhibition at a dose three times that of cisplatin. JM40, the least myelotoxic drug in the system, was approximately 10 times weaker than cisplatin.

With cisplatin as reference agent, the IC_{50}s expressed in μg/ml as listed in Table 1 were used to establish ratios giving the relative toxicities of the platinum analogs (Table 2). The clinical maximum

Table 1. Cytotoxic effect of cisplatin and four analogs on human bone marrow cells

Drug	IC_{50} (moles × 10^{-5})	IC_{50} (μg/ml)
Cisplatin	5.2 ± 0.05	15.6 ± 1.5
Spiroplatin	0.1 ± 0.01	0.4 ± 0.04
Carboplatin	15.2 ± 1.6	56.3 ± 5.9
Iproplatin	8.7 ± 2.0	36.3 ± 8.3
JM40	50.3 ± 14.6	179.5 ± 52.1

IC_{50}: concentration inducing a 50% cell kill. Values: mean ± S.E. expressed in moles and μg/ml.

tolerated doses (MTDs) derived from data in the literature were used to calculate corresponding ratios for clinical and *in vitro* comparison. The comparison of MTD ratios with IC_{50} ratios expressed in moles was slightly less concordant than the comparisons with IC_{50} ratios expressed in μg/ml. Our results showed that the *in vitro* and clinically based ratios correlated amazingly well.

DISCUSSION

The differential sensitivities of normal human myeloid progenitor cells to cisplatin and four analogs were studied with an *in vitro* CFU-assay.

A major focus of interest in the evolution of cancer chemotherapy in recent years has been the development of new drugs with greater tumor specificity and less normal tissue toxicity. Myelosuppression has been recognized as the principal dose-limiting factor for nearly all of the first and second generation drugs [9]. However, most of the toxicity research has focused not on myelosuppression but on the specific organ toxicity associated with the first generation. Thus, investigations into the side-effects of the new platinum compounds have centered on renal toxicity [10].

The experimental model we used appeared attractive for estimating clinical myelosuppression. However, in a previous study, Dodion *et al.* [11] showed that the predictability of the test was insufficient for four anthracyclines.

Incorrect predictions may result from variability in the data due to biotransformation or other pharmacokinetic parameters that may vary from one agent or group to another. In the clinic, thrombopenia is generally observed starting at 100 mg/m^2 of cisplatin, while iproplatin and carboplatin produce a similar effect at the much higher doses of 300 and 400 mg/m^2, respectively. The maximum tolerated doses for spiroplatin and JM40 are 30 mg/m^2 and 1200 mg/m^2. The clinical maximum tolerated dose ratios of the platinum analogs to cisplatin correlate surprisingly well with the corresponding *in vitro* IC_{50} ratios (Table 2). In the case of spiroplatin, the most potent analog, the clinical maximum tolerated dose is higher than would have been predicted from the *in vitro* data. This could be explained by the fact that the *in vitro* Concentration × Time Product (AUC) to which the bone marrow cells were exposed was the same for all drugs, in contrast to the small clinical AUC of spiroplatin in comparison e.g. to cisplatin. Such variation in the kinetics might account for the apparent discrepancy in the case of spiroplatin. Another approach to comparative studies is in fact the use of *in vitro* drug concentrations based on clinical plasma decay curves (AUC).

The CFU assay has been suggested as a biological reference model for the design and interpretation of chemosensitivity studies in the human tumor clonogenic assay [12]. Our results suggest that such experiments seem warranted for cisplatin derivatives.

Results obtained with a leukemic cell line, HL60, differed significantly from those obtained with the bone marrows (results not shown). This would indicate that HL60 is not an appropriate alternative for human bone marrow in these experiments.

In vitro drug concentrations for tumor sensitivity tests could be selected based on their *in vitro* toxicity in the CFU assay as we did in a companion paper [13]. This could indeed minimize the risk of false *in vitro* sensitive results originating from testing at too high doses, and of false *in vitro* resistant results from testing at too low doses [14]. Since our experiments showed a good correlation between IC_{50}s obtained in the CFU assay with normal human bone marrow and the clinical maximum tolerated doses, we

Table 2. Ratios of the in vitro IC_{50}s *of the analogs in* μg/ml *using cisplatin as reference drug and corresponding ratios of the clinical maximum tolerated dose (MTD) with cisplatin as reference drug*

Drug	Ratio based on IC_{50} (μg/ml)	Clinical MTD (mg/m^2)	Ratio based on clinical MTD
Cisplatin	1	100	1
Spiroplatin	0.03	30	0.3
Carboplatin	3.6	400	4
Iproplatin	2.3	300	3
JM40	11.5	1200	12

believe that comparative as well as individual drug studies might be promising for analyzing and predicting myelotoxicity.

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