Alexander D. Guminski · Paul R. Harnett
Anna deFazio

Carboplatin and paclitaxel interact antagonistically in a megakaryoblast cell line – a potential mechanism for paclitaxel-mediated sparing of carboplatin-induced thrombocytopenia

Received: 29 August 2000 / Accepted: 30 January 2001 / Published online: 21 March 2001 © Springer-Verlag 2001

Abstract Purpose: Clinical observation has shown that paclitaxel ameliorates the antiplatelet toxicity of carboplatin when the two drugs are combined, although antitumour activity and white cell toxicity are at least additive. We hypothesized that this is due to an interaction between the two drugs at the level of the platelet precursor. Methods: We measured inhibition of growth of the megakaryoblast cell line MEG-01 following exposure to paclitaxel and carboplatin singly or combined. Drug interaction was assessed by median effect analysis. Results: An antagonistic interaction was observed, and this was most marked at drug concentrations giving a low level of growth inhibition (P < 0.002, sign test). The interaction was not sequence-dependent. There was no significant difference in whole-cell accumulation of platinum or the amount of platinum adducts on DNA following combined treatment in comparison with carboplatin alone. Conclusions: These results provide the first evidence of an antagonistic interaction between paclitaxel and carboplatin in a platelet precursor and provide an explanation for the platelet-sparing effect of the combination of these chemotherapeutic agents. While the mechanisms underlying the interaction described in this report are yet to be fully elucidated, this study provides evidence that the antagonism between paclitaxel and carboplatin in MEG-01 cells is not due to reduced platination of DNA.

Keywords Chemotherapy · Platelets · Drug interaction · Platinum DNA adducts

Introduction

Carboplatin is an effective chemotherapy agent in common malignancies including ovarian and non-small-cell lung carcinomas. Its dose-limiting toxicity is reduction in platelet count, or thrombocytopenia. This effect is dependent on total exposure to the drug which depends on drug clearance and body weight. As carboplatin clearance is primarily dependent on renal function, which in turn can be estimated by simple laboratory tests, an appropriate dose can be determined for each patient using area under the concentration-time curve (AUC) dosing [6, 10]. This ability to appropriately individualize dose prior to first treatment is unusual amongst chemotherapy drugs.

Paclitaxel is also effective against a number of tumour types and the combination of paclitaxel and carboplatin has been extensively investigated [4, 5]. An interesting observation from these studies is that the expected degree of thrombocytopenia is not seen despite the occurrence of significant neutropenia and antitumour effects. The basis of this interaction is unknown. A pharmacokinetic interaction between the two drugs appears unlikely from the data of Kearns et al. [13] and van Warmerdam et al. [21]. These authors compared the degree of thrombocytopenia for measured carboplatin AUC between patients receiving carboplatin combined with paclitaxel and historical values for carboplatin alone. Their analyses show an approximate 60% increase in the carboplatin AUC required to achieve a 50% fall in the platelet count when paclitaxel is given concurrently. We hypothesized that this platelet-sparing
Effect of paclitaxel in combination with carboplatin might indicate an interaction at the level of the platelet precursor. We therefore sought to examine the anti-proliferative effects of paclitaxel and carboplatin, singly and in combination, on a megakaryoblast cell line.

### Materials and methods

#### Cell culture

The megakaryoblast cell line MEG-01 was obtained from the American Type Culture Collection, Rockville, Md. It is a Philadelphia chromosome-positive leukaemic cell line expressing megakaryocyte antigens GpIb/IIa and Factor VIII (but no lymphoid or myeloid surface markers) and with morphological features similar to megakaryocytes as described by Ogura et al. [17]. MEG-01 cells also show differentiation along a megakaryocytic pathway [16]. Cells were grown in RPMI-1640 medium buffered with sodium bicarbonate 5.6% (v/v) and HEPES 10 mM and supplemented with 20% (v/v) fetal calf serum, 1-glutamine 2 mM, sodium pyruvate 1 mM and glucose 4.5 g/l (Astra, North Ryde, Australia). The final pH was approximately 7.2 and the medium was antibiotic-free. All supplements were from Trace Biosciences (Castle Hill, Australia) except where stated. Cultures were maintained in a humidified atmosphere containing 5% CO₂ at 37°C. Cells were free from *Mycoplasma* contamination (Gen-Probe T.C. Rapid Detection System, BioMediq, Doncaster, Australia).

#### Analysis of drug effects on proliferation

Carboplatin (Delta West, Perth, Australia) and paclitaxel (Taxol, Bristol Myers Squibb, Noble Park, Australia) were obtained commercially. This paclitaxel formulation is dissolved in Cremophor EL/ethanol. A vehicle control was prepared using Cremophor EL (Sigma, Steinheim, Germany) plus absolute ethanol, equivalent to the concentration of vehicle in the highest concentration of drug to which cells were exposed. Drugs and vehicle control were diluted in complete medium to obtain final dilutions. Concentration response curves for each agent alone were obtained to define an appropriate concentration range and IC₅₀ (concentration inhibiting proliferation by 50% compared with control). Experiments were conducted by plating logarithmically growing cells at 4x10⁵ cells in 50 μl medium in each well of a 96-well plate and allowing them to grow for 24 h before drug addition. Drug exposure was continuous from the time of addition. Cell proliferation was assessed every 24 h for 144 h using a colorimetric assay (Cell Titer 96 Non-Radioactive Cell Proliferation Assay; Promega, Madison, Wis.) which relies on the conversion by mitochondrial dehydrogenases of a tetrazolium salt to a formazan product [15]. Absorbance was measured on a plate reader (Biorad Microplate Reader Model 550; Biorad, Hercules, Calif.) at 570 nm minus background at 630 nm. Proliferation for each concentration was performed in quadruplicate on each plate and is expressed as the mean of four wells relative to vehicle control.

For the combined-agent experiments, cells were exposed to drug 24 h after seeding and read 48 or 72 h later. Cells on each plate were exposed to five different concentrations of paclitaxel, five of carboplatin and to the same concentrations of each drug combined (see below) in addition to appropriate vehicle controls. The value for each concentration of drug alone or in combination is the mean of four wells. Paclitaxel was added either 3 or 19 h prior to, or 1 or 3 h following, carboplatin.

#### Analysis of drug interaction

Potential drug interactions were analysed using the median-effect method of Chou and Talalay [7]. This model is based on adminis-

tering two drugs in the ratio of their individual IC₅₀ values (or “median effect” dose) and at several fold higher or lower concentrations of each drug. At all drug concentrations, the ratio remains fixed. This analysis is valid if the median-effect plot [log(fraction of cells affected/fraction of cells unaffected) versus log(dose)] is linear, as was found in our experiments for each drug alone and in combination (r-values ranged between 0.948 and 0.998, data not shown).

A further consideration in the model is whether there is any observed interaction between drugs that share a common receptor or pathway (termed mutually exclusive) or whether they interact via distinctive mechanisms (mutually nonexclusive), bearing in mind that a common pathway may occur some way downstream.

From these plots the doses required for a certain level of cell kill for each drug alone and for the drugs in combination can be determined. A combination index (CI) can then be determined at that level of cell kill according to the following equations. For mutually exclusive drugs:

\[
CI = \frac{[\text{drug 1 in combination}]}{[\text{drug 1 alone}]} \quad \frac{[\text{drug 2 in combination}]}{[\text{drug 2 alone}]} 
\]

For mutually nonexclusive drugs:

\[
CI = \frac{[\text{drug 1 in combination}]}{[\text{drug 1 alone}]} + \frac{[\text{drug 2 in combination}]}{[\text{drug 2 alone}]} 
\]

As demonstrated by Chou and Talalay, CI > 1 indicates antagonism, CI = 1 indicates additivity and CI < 1 indicates synergy.

In the situation where the median-effect plots of each individual drug reveal a different slope, as occurred in our experiments (data not shown), then it is not possible to say whether the interaction is mutually exclusive or nonexclusive. We applied the analysis assuming mutual exclusivity as this is the most conservative for identifying antagonism, as can be seen from the two equations above. It is intuitively implicit that two drugs in combination would exert a greater cell kill if they worked via separate pathways than if they utilized the same pathway.

#### Platinum accumulation

MEG-01 cells in logarithmic growth phase were incubated for 3 h in serum-free medium containing either paclitaxel (final concentration 0.105 μM) or paclitaxel vehicle control, or in serum-free medium alone. Carboplatin was then added to a final concentration of 1 mM and the cells, including an untreated control, were incubated at 37°C for a further 4 h. Cells were then harvested, washed twice in ice-cold PBS and finally resuspended in 2 ml PBS. A 0.5-ml aliquot was taken and stored at −20°C for whole-cell platinum and protein determination. The remaining volume was incubated for 1 h at 37°C with 0.1 M ammonium bicarbonate to inactivate non-DNA-bound platinum. DNA was then extracted using a Puregene DNA isolation kit (Gentra Systems, Minneapolis, Minn.). The samples taken for whole-cell determination were subsequently sonicated and aliquots taken for spectroscopic protein determination (Bio-Rad Protein Assay; Bio-Rad Laboratories) and whole-cell platinum measurement. Platinum concentrations were determined using an inductively coupled plasma mass spectrometer (ICPMS; Elan 5000; Perkin Elmer, Sydney; Department of Biochemistry, Royal Prince Alfred Hospital, Sydney, Australia). Experiments were performed in triplicate on two separate occasions. After drug exposure aliquots from drug-treated cells and controls were taken, washed, then resuspended in complete medium and used to seed a colorimetric cytotoxicity assay as above with cell survival measured every 24 h for 72 h following removal from drug exposure.

#### Statistical analysis

All data were statistically analysed using either Microsoft Excel 97 for Windows or SPSS ver. 8. A sign test was used to assess the significance of the differences between the observed CIs. Analysis of