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Original Paper

In Vitro and *In Vivo* Characterisation of Low-resistant Mouse Reticulosarcoma (M5076) Sublines Obtained After Pulse and Continuous Exposure to Cisplatin

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In order to simulate drug resistance observed in the clinic, two cisplatin-resistant cell lines were produced from a murine ovarian reticulosarcoma, M5076 (M5), by pulse (M5/CDDP) and continuous (M5/CDDPc) treatment with *cis*-diamminedichloroplatinum(II)(CDDP). These cell lines showed a similar stable low level of resistance (approximately 3-fold) to CDDP and cross-resistance to carboplatin, iproplatin and the new alkylating agent tallimustine, but not to L-PAM (L-phenylalanine mustard) and BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea). Collateral sensitivity to two inhibitors of topoisomerase II, VP16 (etoposide) and doxorubicin (Dox), but cross-resistance to the topoisomerase I inhibitor, camptothecin, were observed. The two cell lines were also sensitive to 5-fluorouracil. No increase in the level of glutathione or activity of glutathione S-transferase could be observed in resistant cells compared with the parental M5 cells. Total DNA platination immediately after treatment was similar in the parental and resistant cell lines. Repair of total DNA platination, measured after 24 h of recovery, was undetectable in M5 and M5/CDDP cells, but was 33% in M5/CDDPc cells. Initial DNA-interstrand cross-links (DNA-ISC) were six times higher in M5 than in M5/CDDP cells, but 24 h after treatment, both lines had completely repaired this damage. M5/CDDPc cells did not show formation of DNA-ISC at any time after treatment. The two resistant cell lines were tumorigenic when implanted in mice and resistant to CDDP treatment *in vivo*. The CDDP resistant tumours were not cross-resistant *in vivo* to L-PAM, BCNU and Dox, which had been active *in vitro*, nor to tallimustine, which had been cross-resistant *in vitro*. Mechanisms of resistance in M5/CDDP and M5-CDDPc seem to be based on a lower formation of DNA-ISC combined, for the latter cell line, with a higher repair capacity for total DNA platination. **Copyright © 1996 Elsevier Science Ltd**

Key words: cisplatin, resistance, DNA damage, *in vivo*

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INTRODUCTION

cis-DIAMMINEDICHLOROPLATINUM(II) (CDDP) is one of the most effective drugs used in the treatment of human ovarian, testicular, bladder and head and neck cancers [1]. However, as with many other cytotoxic drugs, the development of acquired resistance is a common consequence of therapy, limiting its clinical efficacy [2, 3]. The biochemical and genetic mechanisms of CDDP resistance are not yet fully understood. A number of cell lines with acquired resistance to CDDP have been developed and they have provided model systems for the study of the mechanisms of CDDP resistance [4]. In most cases, resistance mechanisms have been studied in highly resistant (>10-fold) mammalian tumour cell lines in which resistance has been induced by exposing cells continuously to increasing concentrations of CDDP for extensive periods of time [4]. In the clinic, how-

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ever, patients may develop drug resistance at relapse after only a few courses of chemotherapy. Moreover, the tumour resistance observed in patients is not very high (approximately 3- to 6-fold) [5, 6]. Therefore, in terms of the method of induction, the drug resistance induced by a short-term exposure to relatively high doses of antitumour agents may have more clinical relevance than that produced by long-term exposure to low concentrations. Furthermore, study of the mechanisms involved in low level resistance seems more relevant for *in vivo* studies and thereby clinical practice.

In order to simulate the resistance observed in the clinic, we obtained a low CDDP-resistant subline from the ovarian murine reticulosarcoma M5076 (M5) after pulse exposure of the cells to cytotoxic concentrations of CDDP. We compared the level and the supporting mechanism(s) of resistance of this cell line (M5/CDDP) with that of a CDDP-resistant subline (M5/CDDPc) obtained after continuous treatment with non-cytotoxic concentrations of CDDP. We chose this murine tumour cell line because we had already obtained a transplantable tumour resistant to CDDP from this cell line after *in vivo* treatment [7] and characterised the growth and sensitivity to CDDP of this tumour in primary culture [8]. In this report, we describe the cross-resistance pattern of the two independently selected cell lines to other chemotherapeutic agents *in vivo* and *in vitro*.

Glutathione (GSH) levels and the activity of glutathione-S-transferase (GST) were measured and the formation and repair of total DNA platination and DNA-interstrand cross-links (DNA-ISC) were evaluated.

MATERIALS AND METHODS

Chemicals and reagents

RPMI-1640 medium was obtained from Biowhittaker (Walkersville, Maryland, U.S.A.). Glutamine, pyruvate and horse serum were obtained from GIBCO Europe (Paisley, U.K.). DNase I and Nuclease P1 were from Calbiochem Behring (San Diego, California, U.S.A.). NADPH, DTNB (5-5'-dithiobis-2-nitrobenzoic acid), CDNB (1-chloro-2,4-dinitrobenzene), GSH and GSH reductase were from Sigma (St Louis, Missouri, U.S.A.) and metaphosphoric acid from Merck (Darmstadt, Germany). CDDP, carboplatin and VP16 (etoposide) were obtained from Bristol-Myers Squibb Int. Corp. (Syracuse, New York, U.S.A.). L-PAM (L-phenylalanine mustard), iproplatin, BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea), camptothecin and 5-FU (5-fluorouracil) were provided by the National Cancer Institute (Drug Synthesis and Chemistry Branch, DCT, NIH, Bethesda, Maryland, U.S.A.). Dox (doxorubicin) and tallimustine were obtained from Pharmacia Farmitalia-Carlo Erba (Nerviano, Italy). For cell culture treatment, CDDP and iproplatin were dissolved in the medium and incubated at 37°C for 30 min, before addition to the cells, to equilibrate free and serum protein-bound platinum. L-PAM and BCNU were dissolved in 0.5 ml of 0.3 N HCl and then diluted with the medium, and the other drugs were dissolved directly in the medium. For animal treatment, CDDP and Dox were dissolved in 0.9% NaCl and water, respectively, L-PAM was dissolved in 2% 3 N HCl and buffered with 3 N NaOH. BCNU was dissolved in dimethyl sulphoxide and diluted with 0.9% NaCl, 5-FU was dissolved in water.

Cell lines

The M5 cell line was obtained from Talmadge and colleagues [9] and was propagated normally as a monolayer culture in RPMI-1640 medium, supplemented with 15% heat-inactivated horse serum, 1% glutamine and 1% pyruvate at 37°C in an atmosphere with 5% CO_2. Two drug schedules were used to develop resistant cell lines: intermittent and continuous exposure to CDDP (Figure 1). For the intermittent schedule, the M5 cells (3×10^5) were plated in

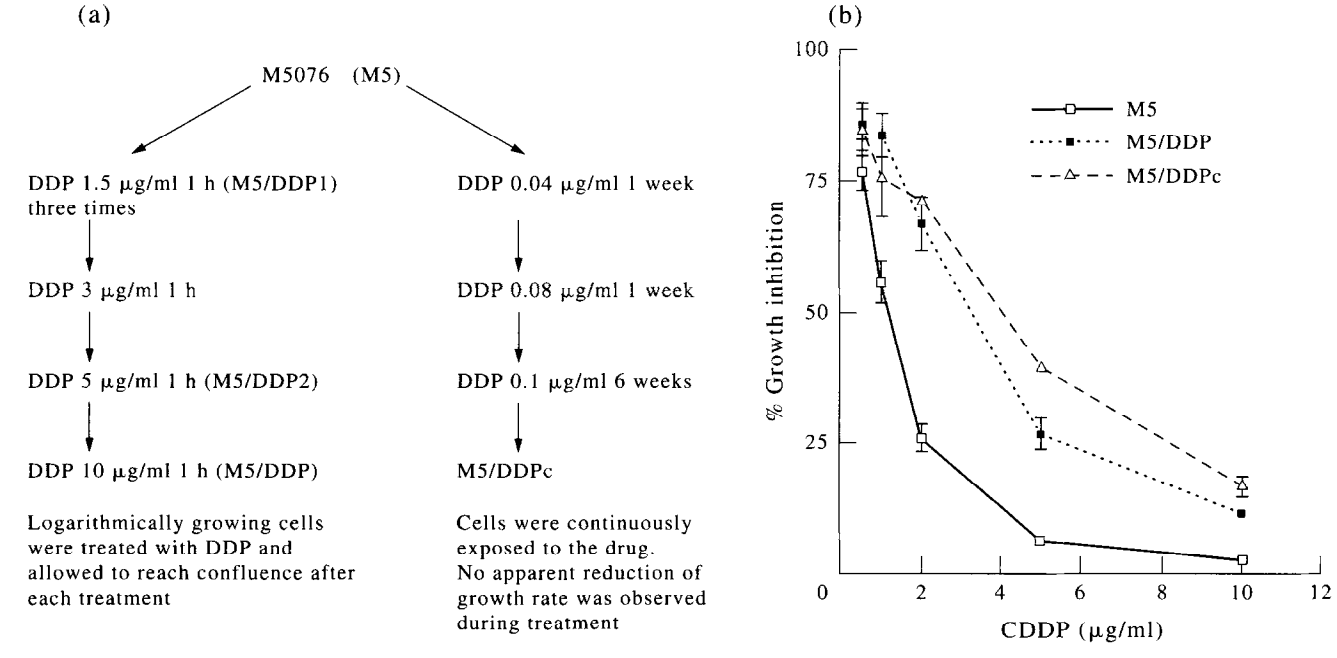

Figure 1. (a) Cisplatin (CDDP) treatment schedules used *in vitro* to obtain the resistant cell lines and (b) growth inhibition caused by CDDP in M5 sublines progressively resistant to CDDP.

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