Correlation of the in vitro cytotoxicity of ethyldeshydroxysparsomycin and cisplatin with the in vivo antitumour activity in murine L1210 leukaemia and two resistant L1210 subclones


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Summary. The cultured murine leukaemia L1210 cell populations used in the present study were derived from L1210 cells that had been grown in vivo. Subclones resistant to sparsomycin (L1210/Sm) or cisplatin (L1210/CDDP) were also developed in vivo. The doubling times of the cultured cell populations were identical. Fractions surviving after drug treatment in vitro were determined by colony formation in soft agar. The results, based on the differential sensitivity of the cell populations to ethyldeshydroxysparsomycin (EdSm) and CDDP, indicated that after a short exposure, cultured L1210/CDDP cells were cross-resistant to EdSm. L1210/Sm cells, however, were not cross-resistant to CDDP. The results obtained in cultured cell populations were confirmed in vivo. CD2fl mice bearing i.p. implants of $1 \times 10^5$ tumour cells were given EdSm or CDDP and a combination of the two agents. Drugs were given once daily every 4 days for 3 doses starting at 24 h after tumour implantation. Treatment of mice bearing L1210/wt leukaemia with combined EdSm and CDDP caused strongly synergistic antitumour activity. In animals bearing the two resistant subclones, however, combined drug treatment did not improve the antitumour activity. The corresponding median survival of mice receiving combined drug treatment was 60 days in each group containing 6 mice bearing L1210/wt, with 4—6 cures being noted; 19 days in animals harbouring L1210/Sm, with 2 cures being recorded among 6 mice; and 11 days in mice bearing L1210/CDDP, with no cure being obtained. The results of this study indicate that the synergism resulting from combined treatment with CDDP and EdSm is a function of the cellular properties of the target tumour-cell populations and is independent of host factors.

Introduction

Cisplatin (CDDP) is a metallic antineoplastic agent that is used in the treatment of cancers such as testicular, ovarian and head and neck tumours. It is thought to act via interand intrastrand cross-linking of duplex DNA [19]. Sparso- mycin (Sm) is a known inhibitor of ribosomal protein synthesis [7, 14]. Pretreatment of L1210 leukemia cells in vitro [27] with Sm strongly enhances the cytotoxic effects of CDDP. In vivo, Sm potentiates CDDP’s antitumour activity when it is given 3—6 h prior to CDDP and results in prolongation of the median survival of mice (>60 days) and in a 66% cure rate [26]. Sm analogues that are more active in vitro than the parent drug have been tested for their antitumour activity in eight in vivo murine tumour models [28]. The most active compounds appeared to be deshydroxy-Sm (dSm), ethyldeshydroxy-Sm (EdSm) and n-pentyl-Sm (pSm).

In vivo potentiation of CDDP’s antitumour activity has been studied for Sm and the three above-mentioned active analogues in s.c. implanted L1210 leukaemia [29]. In this tumour model, CDDP treatment (5 mg/kg) resulted in a 156% treated/control (T/C) value. Treatment with sparsomy-ycin and its analogues alone resulted in an increase in survival for EdSm only (139%). Although Sm itself was incapable of potentiating the antitumour activity of CDDP in this model, two analogues, dSm and EdSm, were active. At a dose of 10 mg/kg, EdSm potentiated CDDP’s antitumour activity by 2.8 times. pSm, the third analogue, showed no potentiation of the antitumour activity of CDDP in this tumour model.

On the basis of these results, EdSm was chosen for further preclinical studies on the synergism between sparsomycins and CDDP. To investigate the relationship between the results of combined drug treatment in vivo and the drug sensitivity of tumour cells in vitro, we derived two resistant sublines from L1210 murine leukaemia. Our further goal was to examine the correlation between in vitro sensitivity testing and in vivo antitumour activity. The outcome of this investigation might facilitate further
Fig. 1 A–D. In vitro chemosensitivity of L1210 leukaemia (○) and two drug-resistant subclones, L1210/Sm (●) and L1210/CDDP (■), exposed to EdSm and CDDP. A, B Effect of 1 h exposure. C, D Effect of continuous exposure. A, C Cytotoxicity of EdSm. B, D Cytotoxicity of CDDP. Data represent mean values for experiments performed in triplicate.

Materials and methods

Cell culture. Murine L1210 leukaemia cells (L1210/wt) were kindly supplied by Dr. G. Atassi (Institute Jules Bordet, Laboratory for Experimental Chemotherapy, Brussels). Cells were maintained in logarithmic growth as suspension cultures in RPMI 1640 medium containing 10% fetal bovine serum and antibiotics. Cells were grown in a humidified atmosphere containing 5% CO₂ at 37°C.

Drug-resistant sublines. The murine L1210 cisplatin-resistant subclone (L1210/CDDP) was kindly supplied by Dr. G. Atassi and was developed in vivo. We established another subclone of mouse tumour cell line L1210 with acquired resistance to sparsomycin by repeated in vivo studies on the synergistic antitumour effects of EdSm on other cytostatic agents.