Characterization of cisplatin-induced DNA damage in human bladder tumour cell lines

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Cisplatin [cis-diamminedichloroplatinum (II)] is one of the most effective single agents employed in the treatment of carcinoma of the urinary bladder (Harker & Torti, 1983). A major obstacle in the clinical use of this agent is, however, the development of resistance (Frei et al., 1985). Since DNA is widely believed to be the critical target for the cytotoxic effect of platinum complexes (Roberts & Thomson, 1979) we have compared DNA damage and repair in two human bladder tumour cell lines with differential sensitivities to cisplatin.

The continuous cell lines T24 and RT112 were both derived from transitional cell carcinomas of the human bladder and were grown as monolayers using conditions described elsewhere (Hepburn et al., 1985). The T24 cells were almost twice as sensitive (IC_{50} 7.6 µg/ml) as RT112 cells (IC_{50} 14.4 µg/ml) after 1 h exposure to a cytotoxic range of concentrations of cisplatin. DNA–DNA interstrand cross-links (ISL) were quantified by alkaline filter elution (Kohn et al., 1981) as a proteinase-resistant transferase expression in hybrid cells but is not the case for aryl hydrocarbon hydroxylase or glutathione S-epoxide transferases (Benedict et al., 1972; Silberstein & Shows, 1982). Further work is in progress to test these possibilities.

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Abbreviations used: cisplatin, cis-diamminedichloroplatinum (II); IC_{50}, concentration reducing cell survival to 10% of the control after a 1 h exposure; ISL, DNA–DNA interstrand cross-links.
Drug resistance *in vitro*: interactions between X-irradiation, etoposide and vincristine

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Reduced response rates to chemotherapy have been observed in a number of human 'solid' tumours after prior treatment, not only with chemotherapy, but also with radiotherapy (Holland et al., 1980; Price & Hill, 1981; Thigpen et al., 1981; Young et al., 1982). In an effort to discover the underlying basis for these clinical observations a human tumour continuous cell line (HN-1), derived from an epithelioid carcinoma of the tongue, was treated with fractionated X-irradiation in *vitro* and the resultant sub-line (HN-1/DXR) was shown to express resistance to etoposide (VP-16-213) and vincristine (Hill & Bellamy, 1984).

VP-16-213, a semi-synthetic derivative of podophyllotoxin, has a broad spectrum of clinical anti-tumour activity, proving one of the most active single agents in clinical use (Watanabe et al., 1983). Its mechanism of cytotoxicity appears to involve DNA strand breakage (Loike & Horwitz, 1976; Long et al., 1984; Wozniak & Ross, 1983) and subsequent chromosomal aberrations (Huang et al., 1973) brought about by a poisoning of DNA topoisomerase II (Ross et al., 1984).

Vincristine has also shown considerable clinical anti-tumour activity in combination with other drugs (O'Dwyer et al., 1985). Its mechanism of cytotoxicity appears to involve DNA strand breakage (Loike & Horwitz, 1976; Long et al., 1984; Wozniak & Ross, 1983) and subsequent chromosomal aberrations (Huang et al., 1973) brought about by a poisoning of DNA topoisomerase II (Ross et al., 1984).

The following parameters have been studied: drug uptake, glutathione levels, and extent of DNA damage after drug treatment.

Drug responses were established by assessing cell survival by colony formation in soft agar (Courtenay & Mills, 1978). $IC_{50}$ values for the HN-1, HN-1/DXR and HN-1/VP-2 cells were 3.2, 5.0 and 9.7 $\mu M$ respectively after a 1 h exposure to VP-16-213. The order of resistance expressed by the HN-1/DXR and HN-1/VP-2 cells to vincristine was 2.8 and 3.4 respectively compared with the HN-1 line after exposure to 8 nm-vincristine for 24 h.

Drug uptake studies using $[^3H]$VP-16-213 at 3.1 $\mu M$ for a 30 min exposure have shown no significant differences between the three lines, with values of 18.3 $\pm$ 1.9, 20.1 $\pm$ 0.5 and 19.1 $\pm$ 4.6 pmol/mg protein for the HN-1, HN-1/DXR and HN-1/VP-2 cells respectively. However, using $[^3H]$vincristine at 1 $\mu M$ for 30 min there was reduced uptake in the drug-treated sub-line (198 $\pm$ 14 pmol/mg of protein) compared with the parental cells (300 $\pm$ 2 pmol/mg of protein) and the X-irradiation-treated (387 $\pm$ 14 pmol/mg of protein) sub-line.

Levels of non-protein sulphydryl compounds have been estimated and shown not to differ significantly between the sub-lines, total cellular contents being 55 $\pm$ 5, 57 $\pm$ 2 and 47 $\pm$ 2 nmol/mg of protein for the HN-1, HN-1/DXR and HN-1/VP-2 cells respectively. The drug-treated sub-line also showed no alteration in glutathione S-transferase activity compared with the parental line with activities of 249 $\pm$ 24 and 234 $\pm$ 17 nmol/mg per min respectively.

Alkaline elution studies have revealed a reduction in total DNA breakage and estimated single-strand breakage in the drug treated sub-line (HN-1/VP-2) compared with the parental line after exposure to equimolar concentration of VP-16-213. Rad-equivalent values for total DNA breakage are 346 $\pm$ 35 and 461 $\pm$ 11, and for single-strand breakage are 625 $\pm$ 131 and 1252 $\pm$ 309 for HN-1/VP-2 and HN-1 cells respectively (8.5 $\mu M$-VP-16-213, 1 h).

These data indicate that different mechanisms of resistance may be implicated in these differently derived resistant cell lines. Further studies using isolated nuclei should reveal whether the alteration in DNA damage in the drug-treated sub-line is independent of drug uptake through the plasma membrane, a finding that would suggest that resistance to VP-16-213 in these cells may be associated with reduced activity of DNA topoisomerase II (Pommier et al., 1985).

Abbreviation used: $IC_{50}$, drug concentration required to reduce cell survival to 50% of the control.