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SHORT COMMUNICATION

Exceptional sensitivity of testicular germ cell tumour cell lines to the new anti-cancer agent, temozolomide

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Summary Metastatic testicular germ cell tumours are cured in approximately 85% of patients using cisplatin-based combination chemotherapy. Patients who fail to respond have a poor prognosis, and there is a need for more effective treatments for cisplatin-resistant disease. In this study, it is shown that two of four cell lines derived from human non-seminomatous testicular germ cell tumours are exceptionally sensitive to temozolomide, a new imidazotetrazine which can cross the blood-brain barrier in mice. In addition, three pairs of cisplatin-resistant sublines show little cross-resistance to temozolomide. These data suggest that temozolomide might have activity against non-seminomatous testicular germ cell tumours which have relapsed following cisplatin-containing chemotherapy, and could have a role in the treatment of patients with metastatic lesions in the brain.

Keywords: testicular germ cell tumour; temozolomide; chemotherapy

Despite the high cure rate obtained with combination chemotherapy of non-seminomatous testicular germ cell tumours, the management of patients with an adverse prognosis at presentation, or of those who fail to respond to first-line chemotherapy, remains a therapeutic challenge (Horwich *et al.*, 1993). Most experimental protocols aimed at these poor-risk groups, which constitute about 15% of the patient population, involve intensification of chemotherapy with agents already known to be active against the disease. It may be that the addition to these regimens of a new drug with a different molecular mechanism of action would have a positive impact on the outcome of treatment.

Cell lines derived from human non-seminomatous testicular germ cell tumours are highly sensitive to cisplatin (Pera *et al.*, 1987*a*; Walker *et al.*, 1987). The pattern of sensitivity observed *in vitro* appears to reflect the clinical response, as testis tumour cells are 3-10 times more sensitive to cisplatin, bleomycin and etoposide than bladder cancer cells, comparing IC_{50} values (Masters *et al.*, 1993). These cell lines are also highly sensitive to another class of DNA-damaging agent: compounds which can alkylate the O^6 position of guanine (Walker *et al.*, 1992). The mutagenic consequences of O^6 alkylation of guanine are well described. In addition, some mammalian cells are acutely sensitive to the cytotoxic effects of compounds which generate this lesion (reviewed in Roberts, 1978).

Temozolomide, which undergoes chemical decomposition to yield a monofunctional alkylating agent capable of binding to the O^6 position of guanine, is a new anti-cancer agent which is less toxic than mitozolomide (Newlands *et al.*, 1992) and which has the ability to cross the blood-brain barrier in mice. We compared the sensitivities to temozolomide of a series of human bladder and testicular germ cell tumour cell lines and three pairs of parent and cisplatin-resistant sublines.

Materials and methods

The origins of the cell lines used in this study are shown in Table I. All cell lines were maintained routinely under identical conditions as monolayers in 25 cm^2 tissue culture flasks (Nunc, Gibco, Paisley, UK) using alpha minimum essential tissue culture medium (αMEM) supplemented with a single batch of 5% heat-inactivated fetal calf serum (Imperial Labs) and 2 mM L-glutamine (Flow, Irvine, UK) in a humidified atmosphere of 5% carbon dioxide in air at 36.5°C. The cells were used over a restricted range of ten passages to minimise changes that might occur during long-term culture.

Clonogenic assay

Exponentially growing cells were detached using 0.05% trypsin (Difco 1:250; Difco, London, UK) in an aqueous solution of 0.016% ethylenediamine tetraacetic acid disodium salt (EDTA; BDH Chemicals, Poole, UK) and were plated in 5 cm dishes containing 5 ml of prewarmed and gassed medium at a plating density designed to produce approximately 200 colonies per plate in the untreated controls. Following a 24 h incubation to permit attachment and the resumption of exponential growth, the medium was replaced either with fresh medium alone (in quintuplicate) or containing 0.5% dimethyl sulphoxide (DMSO; Sigma, Poole, UK) or a range of temozolomide (Aston Molecules, UK) concentrations (in triplicate for each concentration).Temozolomide was dissolved in DMSO and the stock solution subsequently added to culture medium. The final volume of DMSO did not exceed 0.5%, a non-toxic concentration. Preliminary experiments identified the range of cytotoxic concentrations, and at least three concentrations which fell in the exponential region of the dose-response curve were selected. After a further 9-15 days' culture, colonies were fixed in methanol (BDH) and stained with 10% Giemsa (BDH). Colonies containing a minimum of 50 cells were counted using a binocular dissecting microscope. The mean colony-forming ability was expressed as a percentage of the untreated controls and computed using least-squares regression analysis on the straight portion of the dose-response plot. The drug was tested against each cell line in at least two further experiments.

Results

The toxicity of temozolomide to four testis tumour and three bladder cancer cell lines was measured (Figure 1a). Two of

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Table I Origins and characteristics of the cell lines used in this study

Cell line designation	*Histopathological type*	*Previous treatment*	*ATase level[a] (fmol mg^{-1} protein)*	*Temozolomide mean IC_{50} (μg ml^{-1})*	*References*
SuSa	NSTGCT primary	None	206	23.2	Hogan *et al.* (1977)
SuSa-CP	Cisplatin-resistant subline		470	50.5	Walker *et al.* (1990)
833K	NSTGCT (abdominal metastasis)	Chemotherapy[b]	ND	27.6	Bronson *et al.* (1980)
GCT27	NSTGCT primary	None	3.3	0.54	Pera *et al.* (1987*b*)
GCT27-CP	Cisplatin-resistant subline		ND	0.72	Kelland *et al.* (1992)
GCT44	NSTGCT (lymph node metastasis)	Chemotherapy[c]	ND	5.8	Pera *et al.* (1987*b*)
RT112	TCC primary	None	387	24.2	Masters *et al.* (1986)
RT112-CP	Cisplatin-resistant subline		301	45.9	Walker *et al.* (1990)
MGH-U1 (T24)	TCC recurrence	None	603	24.5	Bubenik *et al.* (1973)
HT1376	TCC primary	None	506	30.3	Rasheed *et al.* (1977)

NSTGCT, non-seminomatous testicular germ cell tumour; TCC, transitional cell carcinoma of the bladder; ND, not done. [a]From Walker *et al.* (1992). [b]Methotrexate, actinomycin D, cyclophosphamide. [c]Cisplatin, etoposide, bleomycin, vinblastine, methotrexate, doxorubicin, actinomycin D, cyclophosphamide.

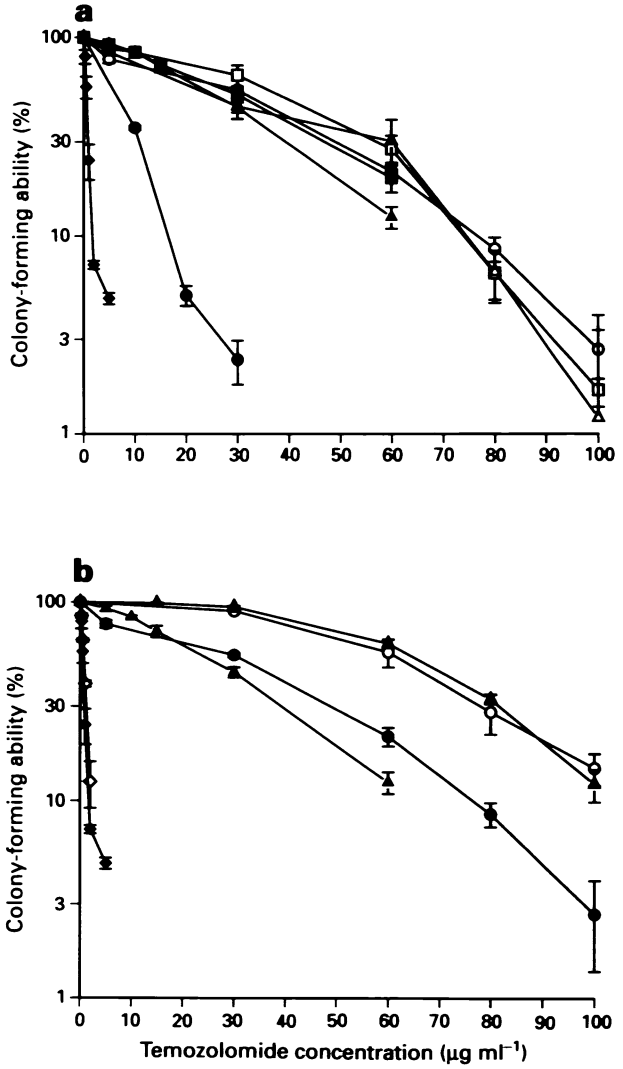

Figure 1 Dose–response curves of testis and bladder cancer cell lines to temozolomide. Cells plated at clonal density were exposed to a range of concentrations of temozolomide, and after a further 9–15 days' culture the colony-forming ability of the treated cells was compared with that of the untreated controls. (**a**) Data for four testis: (●, GCT44; ▲, SuSa; ■, 833K; ◆, GCT27) and three bladder (○, RT112; △, MGH-U1; □, HT1376) cancer cell lines. (**b**) Data for three pairs of parent (▲, SuSa; ◆, GCT27; ●, RT112) and cisplatin-resistant sublines (△, SuSa-CP; ◇, GCT27-CP; ○, R112-CP). Survival for each drug dose was estimated from triplicate dishes, and each experiment was repeated at least twice. The error bars show the standard error of the mean of the separate experiments.

the four testis tumour lines, GCT27 and GCT44, were far more sensitive to temozolomide than the other cell lines. GCT27 was derived from a primary tumour in a patient who had not been treated with chemotherapy. In contrast, GCT44 was derived from a metastatic lesion in a patient who had previously been treated and subsequently died of his disease. GCT44 represents an aggressive biological variant of teratoma (Pera *et al.*, 1987*b*). Comparing the temozolomide sensitivity of three of these lines with their cisplatin-resistant sublines, the relative cross-resistance to temozolomide ranged from 1.3 for GCT27 to 2.2 for SuSa (Figure 1b and Table I), comparing the mean concentrations reducing colony-forming ability by 50% (IC_{50}). The sublines were derived by continuous *in vitro* exposure to cisplatin, and exhibit a 4- to 6-fold resistance to cisplatin, comparing doses that reduce clonogenic cell survival by 50%.

Discussion

Two testis tumour cell lines were shown to be exceptionally sensitive to temozolomide. This finding extends our observation that testis tumour cell lines are particularly sensitive to *N*-nitroso-*N*-methylurea (MNU) and mitozolomide (Walker *et al.*, 1992). This earlier study also demonstrated that GCT27 is more sensitive than the other testis tumour lines studied, with IC_{50} values to MNU and mitozolomide of 1.2 and 0.3 μg ml^{-1}, compared with 12.2 and 1.3 μg ml^{-1} for SuSa (testis) and 56.9 and 4.5 μg ml^{-1} for RT112 (bladder). The greater sensitivity of GCT27 to temozolomide, mitozolomide and MNU may be related to its low levels of O^6-alkylguanine–DNA alkyltransferase activity (see Table I). Low levels of this enzyme may result in higher levels of O^6 alkylation, a potentially toxic DNA lesion.

The exceptional sensitivity of the two testis lines to temozolomide is one reason for testing this new drug in the clinic against testis tumours. The major limitation to the successful treatment of these patients is the presence of cisplatin-resistant disease. Therefore, a further rationale for testing this new agent is the observation that in three independent pairs of cell lines there is relatively little change in the sensitivity to temozolomide in the cisplatin-resistant derivatives. A third reason for testing this agent is its clinical activity against brain tumours (Newlands *et al.*, 1992). Brain metastases occur in 8–15% of patients with testicular tumours, almost always associated with relapse at other sites or as a terminal event (Raina *et al.*, 1993). Temozolomide may provide a more effective treatment for testis tumours which have metastasised to the brain.

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