Eur J Cancer, Vol. 26, No. 6, pp. 742–747, 1990.
Printed in Great Britain

0277–5379/90$3.00 + 0.00

Development and Characterization of Cisplatin-resistant Human Testicular and Bladder Tumour Cell Lines

M. Claire Walker, Susan Povey, Jennifer M. Parrington, Peter N. Riddle, Ruth Knuechel and John R.W. Masters

Cisplatin-resistant cells were derived *in vitro* from a human bladder carcinoma cell line (RT112) and a testicular tumour cell line (SuSa) by continuous exposure to increasing concentrations of cisplatin for 14 and 11 months, respectively. Both resistant cell lines had a four-fold level of resistance relative to their parental cell lines, comparing the cisplatin concentration to inhibit colony forming ability by 70%. These levels of resistance were retained in the absence of cisplatin for at least 3 months. In each case, four-fold fewer micronuclei were produced in the resistant lines by the same cisplatin concentrations. Cross-resistance to carboplatin and methotrexate was observed in both resistant cell lines, but neither line was resistant to doxorubicin. Isozyme and DNA analysis with hypervariable probes confirmed the origin of each resistant cell line from its parental line. Population doubling times and intermitotic times were similar in each of the pairs of cell lines. Karyotyping showed that the resistant cell lines had gained and lost marker chromosomes, but there were no changes common to both resistant cell lines.
Eur J Cancer, Vol. 26, No. 6, pp. 742—747, 1990.

INTRODUCTION

CISPLATIN is the most active single agent in the treatment of advanced testicular germ cell tumours and transitional cell carcinomas of the bladder. Combination regimens containing cisplatin cure most patients with disseminated testicular tumours [1], and induce complete responses for 2–4 years in about a fifth of patients with advanced bladder cancer [2]. Efficacy is limited by intrinsic or drug induced resistance. Cisplatin resistance has been induced *in vitro* in several human and rodent cell lines, but not in human bladder and testicular tumour cell lines. These cell lines reflect the patterns of sensitivity of the tumour to cisplatin and other anti-cancer agents [3, 4]. We report the induction of cisplatin resistance in a bladder and a testicular tumour cell line and characterization of the resulting sublines.

Correspondence to: M. Claire Walker, Cancer Research Campaign, 2 Carlton House Terrace, London SW1Y 5AR, U.K.
M.C. Walker and J.R.W. Masters are at the Department of Pathology, Institute of Urology, St Paul's Hospital, London WC2, S. Povey and J.M. Parrington are at the MRC Human Biochemical Genetics Unit, Galton Laboratory, University College, London, P.N. Riddle is at the Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2, U.K. and R. Knuechel is at the Department of Pathology, RWTH Aachen, Neues Klinikum, Aachen, F.R.G.

MATERIALS AND METHODS

Cell lines

RT112 was established in this laboratory from a well-differentiated transitional cell carcinoma of the bladder in an untreated patient [5]. SuSa was provided by Dr B. Hogan of the Imperial Cancer Research Fund, Mill Hill Laboratories, London, and was derived from a malignant testicular teratoma in an untreated patient [6].

Drugs

Cisplatin (Farmitalia Carlo Erba), containing sodium chloride (final concentration 0.9%) and carboplatin (Sigma) were dissolved initially in distilled water. Methotrexate (Lederle), doxorubicin (Farmitalia Carlo Erba) and bleomycin (Lundbeck) were dissolved in Dulbecco's phosphate-buffered saline (PBSA), and etoposide (Bristol-Myers) was added directly to medium. For chemosensitivity assays, drugs were made up immediately before use except for carboplatin, which was stored in solution at −20°C. For induction of resistance, small amounts of cisplatin were required frequently and were stored at −20°C.

Cell culture

The cell lines were maintained under identical culture conditions. Monolayers were grown in 25 cm^2 flasks (Nunc) in RPMI 1640 medium (Gibco) supplemented with 5% heat-

inactivated foetal calf serum (Gibco) and 2 mmol/l L-glutamine (Flow) at 36.5°C in a humidified atmosphere of 5% CO_2 in air. Cells were subcultured in an aqueous solution of 0.05% trypsin (Difco, 1:250) and 0.016% EDTA (BDH). Parent cell lines were used over a range of 10 passages to minimise any changes occurring as a result of long-term culture.

Development of resistance to cisplatin

RT112 cells, growing exponentially, were exposed initially to a cisplatin concentration that inhibited colony formation by 30% in a continuous exposure assay (80 ng/ml) [3]. Because of low toxicity against RT112 cells, SuSa was exposed initially to an IC_{90} concentration, 50 ng/ml. When the cells appeared to be growing through these concentrations of cisplatin, the concentrations were increased to 100 and 62.5 ng/ml, respectively. The cell lines were exposed to increasing cisplatin concentrations for 14 months (RT112) and 11 months (SuSa). Samples of cells were frozen periodically in liquid nitrogen to provide a stock in case of contamination.

Cytotoxicity assays

After continuous exposure to cisplatin, the sublines (RT112-CP and SuSa-CP) were maintained for 3 months in the absence of drug and resistance was assessed every month. The sensitivities of parent and resistant cell lines were compared in parallel experiments after a 1 h exposure to a range of seven cisplatin concentrations in a colony forming assay [7]. Cytotoxicity assays were repeated at least three times and the results are expressed as means and S.E.

Growth characteristics of parent and resistant cell lines

Population doubling-times and plating efficiencies on plastic were measured as described [5]. Intermitotic times were recorded by timelapse cinemicroscopy of exponentially growing cells with an inverted microscope fitted with a 37°C chamber and 5% CO_2 supply. Photographs were taken (fifteen frames per h) with Kodak AHU film. Cells were followed for one to two generations [8]. The proportion of DNA-synthesising cells was established by exposure of exponentially growing cells for 30 min to 10 μmol/l 5-bromo-2'-deoxyuridine (Sigma) at 37°C [9] and immunocytochemical staining [10]. The proportion of positively stained nuclei was obtained by counting a total of 1000 cells per slide on duplicate slides. This was repeated at least twice and the result averaged.

Isozyme analysis

Cells were prepared [5] and at least five polymorphic enzymes were typed by horizontal starch gel electrophoresis [11].

DNA analysis

High molecular weight DNA was prepared from RT112 and RT112-CP [12]. The probes λMS1 and pλg3, derived from locus-specific hypervariable sequences [13] were provided by ICI Diagnostics as cut-out inserts. Random oligonucleotide priming [14] was used to label 50 ng probe with [α-^{32}P]dCTP (Amersham). DNA (5 μg) from each cell line was digested with *Hin*fI (Anglian Biotechnology, Colchester) and electrophoresed for 24 h at 1.3 V/cm on 0.8% agarose. The buffer was 0.04 mol/l Tris acetate/0.001 mol/l EDTA. Transfer to Gene Screen Plus (Du Pont Biotechnology) and hybridization conditions were as described [15], except that the filter was washed to a final stringency of 0.2 × sodium saline citrate/0.1% sodium dodecyl sulphate at 65°C.

Karyotyping

Metaphase spreads were prepared and stained by standard methods. Modal chromosome numbers were obtained by counting 15 orcein-stained cells from each cell line. At least 4 G-banded cells were photographed and fully analysed for each cell line.

Mycoplasma screening

Parent and resistant cell lines were regularly screened with Hoechst 33258 (Sigma) for mycoplasma contamination [16]. All tests were negative.

Micronucleus assays

Cells (4×10^5 RT112, RT112-CP, SuSa-CP; 6×10^5 SuSa) were plated into 3.5 cm Petri dishes (Nunc) containing a sterile coverslip and 3 ml prewarmed and gassed medium. After 48 h at 36.5°C, medium was removed and replaced with medium alone (controls) or containing cisplatin. After a 1 h exposure to cisplatin, cells were washed three times with medium, then incubated in fresh medium for a further 48, 72, 96 or 120 h. At each time point, two dishes per concentration and control were rinsed in PBSA, fixed for 10 min in methanol/acetic acid (3:1), rinsed again, and air-dried. Micronuclei were stained with Hoechst 33258 (1 μg/ml for 5 min) and counted by fluorescence microscopy. The criteria for identifying micronuclei of Heddle *et al.* [17] were used. Coverslips were randomised and counted blind and at least 1000 nuclei were scored per coverslip. The number of micronuclei per thousand nuclei was expressed as a percentage and the value for the two coverslips per concentration or control was averaged. Percentage micronuclei in controls (background levels) were subtracted from treated values. The results are the means of two to four experiments per cell line.

Cross-resistance

Cross-resistance to chemotherapeutic drugs routinely used in the treatment of testis and bladder cancer and with different modes of action was investigated. The responses of parental and resistant cell lines to the inducing agent, cisplatin, and to carboplatin, doxorubicin, methotrexate, etoposide and bleo-

Table 1. Cisplatin sensitivity and growth characteristics of parent and resistant cell lines, mean (S.E.)

	RT112	RT112-CP	SuSa	SuSa-CP
IC_{70}* (μg/ml)	4.7 (0.4)	19.1 (0.9)†	1.1 (0.1)	4.6 (0.2)†
Population doubling-time (h)	21.6 (0.1)	21.5 (1.5)	27.7 (1.3)	29.8 (0.8)
Colony-forming efficiency (%)	55.3 (6.4)	51.0 (3.5)	7.6 (2.0)	16.6 (2.1)‡
DNA synthesising cells	31.6 (2.1)	41.4 (2.0)§	64.5 (1.2)	49.5 (3.3)§
Intermitotic time (h)	21.0 (1.1)	22.4 (0.5)	22.8 (2.1)	19.3 (0.5)

*From individual linear regression of three to five experiments per cell line.

Statistical significance assessed using Student's unpaired *t* test.

Unpaired *t* test of parent vs. resistant cell line: †$P < 0.001$, ‡$P < 0.02$ and §$P < 0.05$.

Table 2. Isozyme profiles of parent and resistant cell lines

Cell line	Enzyme loci								
	PGM1	PGM3	GOTm	GOTs	ESD	ADA	ACP1	GLO	PGP
RT112	0	ba	2–1	1	2–1 (1>2)	1	B	2–1	1
RT112-CP	1	ba (b>a)	2–1	1	2–1 (2>1)	1	B	2–1	1
SuSa	1	a	—	—	2–1	1	—	2	—
SuSa-CP	1	a	1	1	2–1	1	—	2	1

PGM1 and PGM3 = first and third loci of phosphoglucomutase; GOTm and GOTs = mitochondrial and soluble glutamate-oxaloacetate transaminase; ESD = esterase D; ADA = adenosine deaminase; ACP1 = first locus of acid phosphatase; GLO = glyoxalase; and PGP = phosphoglycolate phosphatase.
> indicates one allele expressed to greater degree.

mycin were compared in an MTT assay [18]. Cells were plated in 150 μl medium into rows 2–12 of a 96-well microwell plate (Nunc) at a density that would result in an absorbance of 1–1.5 absorbance units in sub-confluent control wells 7 days later; medium alone was placed in row 1 as a blank. After 24 h at 36.5°C, 50 μl fresh medium was added to rows 1 and 2 (blanks and controls), and 50 μl medium containing a range of drug concentrations was added to rows 3–12. After a further 6 days, 50 μl tetrazolium dye (final concentration 400 μg/ml) was added to every well and the plates incubated at 36.5°C for 3 h. Medium was aspirated gently. Dimethyl sulphoxide (100 μl/well) was added to solubilise the purple formazan crystals, and the plates were shaken gently. Absorbance at 540 nm was measured on a plate-reading spectrophotometer. Percentage survivals relative to controls were calculated.

RESULTS

Development of resistance

After 14 months' exposure to gradually increasing concentrations of drug, RT112-CP was able to grow continuously in 3.5 μg/ml cisplatin. After 11 months' exposure to increasing concentrations of drug, SuSa-CP was able to grow in 300 ng/ml cisplatin.

Cytotoxicity assays

RT112-CP and SuSa-CP were both four times more resistant than the parent cell line when IC_{70}s were compared (Table 1).

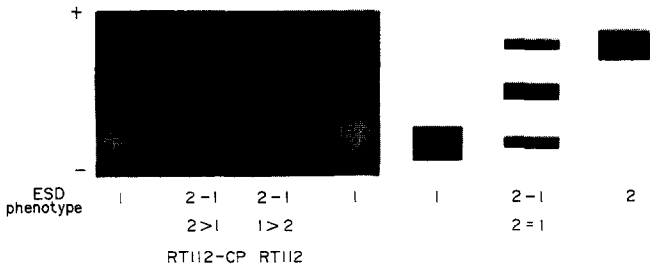

Fig. 1. Starch gel stained for ESD. Lanes 1 and 4 control ESD*1. Lane 3 shows predominance of ESD*1 in RT112 and lane 2 shows predominance of ESD*2 in RT112-CP. The right had diagram is an idealised representation of homozygous and heterozygous ESD phenotypes.

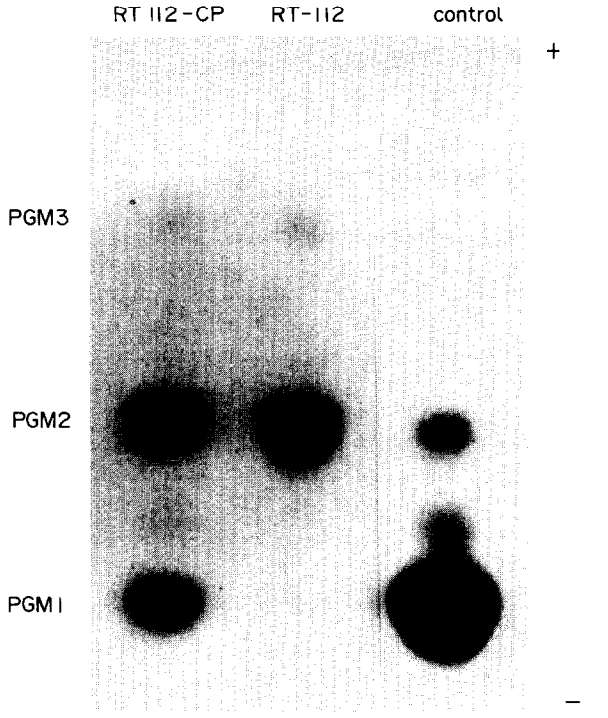

Fig. 2. Starch gel stained for PGM. Lane 3 = control PGM1; lane 2 shows absence of PGM1 in RT112 and lane 1 shows its presence in RT112-CP.

These levels of resistance were stable when RT112-CP and SuSa-CP were maintained in the absence of cisplatin for 3 months.

Characteristics of parent and resistant cell lines

Population doubling-times and intermitotic times were similar in parent and resistant cell lines (Table 1). Plating efficiency increased significantly in SuSa-CP. The proportion of DNA-synthesising cells in the resistant sublines differed significantly from those of the parent line, increasing in RT112-CP but decreasing in SuSa-CP.

Isozyme analysis

The isozyme profiles of SuSa and SuSa-CP were identical at loci tested, but those of RT112 and RT112-CP differed (Table 2). Both cell lines were heterozygous for esterase D but the relative proportions of the isozymes had altered, such that the product of the ESD*1 allele was predominant in RT112 whereas in RT112-CP the product of ESD*2 was predominant (Fig. 1). The most striking change was the appearance of PGM1 in RT112-CP; this was not detected in the parental line (Fig. 2). Other isozyme patterns, including the uncommon heterozygous pattern of GOTm, were unchanged in RT112-CP.

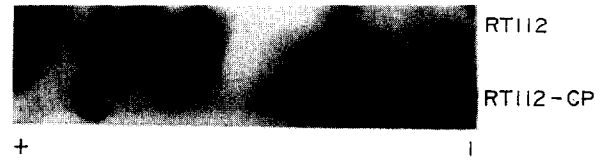

Fig. 3. Autoradiograph of RT112 and RT112-CP DNA hybridised with λMS1 hypervariable probe, showing identical positions for each of two bands and indicating common origin for the two cell lines.

Table 3. Chromosome rearrangements, monosomies and trisomies in parent and resistant cell lines

Modal No.	RT112 47	RT112-CP 43	SuSa 53	SuSa-CP 52
Chromosome		del 1p	1p−, 1q+	1p−, 1q+
1			1q−	1q−
2				
3	3p−	3p−		
4		−4		
5			5p+	5p+
6	6q+		+6q+	+6q+
7			7q−	7q−
8	i8q	i8q		
9	9q+			+9
10			+10	
11		11p+		
12	12q+		+12, +i12p	2 × i12p
			+? 12q−	12p−
13	−13	13q−	13q+	−13, 13q+
14	14p+			−14
15		15p+	−15, 15q+	−15, −15
16		16p+		
17	17p+			
18		−18, −18	+18	−18
19		−19		
20	+20	20p+	+20	
21	i21q	i21q	−21	
22				
X				Xp+
Y	—	—		
Unidentified markers	1 (?del13q)	1 (?3q−)	1 (?8q−)	3 (?8q−)

DNA analysis

Because of the changes in isozyme profile in RT112-CP, a further check on its identity was carried out with two hypervariable DNA probes, λMS1 and pλg3. RT112 and RT112-CP gave identical heterozygous patterns with these probes, which confirmed their common origin (Fig. 3).

Karyotypes

Karyotypes differed between parent and resistant cell lines. The modal number for RT112 was 47, but for RT112-CP was 43 (Table 3). Of the 10 marker chromosomes in RT112, only 3 were retained in RT112-CP; 6 new ones were gained and 5 parental markers were replaced by normal chromosomes in RT112-CP. No homogeneously staining regions or double minutes were seen. The karyotypes of both RT112 and RT112-CP were consistent in all the cells analysed. To confirm that the karyotypic changes were not simply due to repeated passaging during development of resistance, the parental line was subcultured until its passage number reached that of the resistant subline. The karyotype of RT112 was stable over the number of passages that RT112-CP had undergone while in cisplatin. Karyotypic changes in SuSa-CP were not as extensive as in RT112-CP. The modal numbers of SuSa and SuSa-CP were similar, and they had several markers in common (Table 3). RT112-CP and SuSa-CP did not have any markers in common.

Micronucleus production

Preliminary experiments showed that micronucleus production in these cell lines reached a peak 72 h after cisplatin exposure. Increasing the cisplatin concentration led to increased production of micronuclei (Table 4). In a comparison of numbers of micronuclei induced by equimolar cisplatin concentrations (8 μg/ml) for RT112 and RT112-CP; 2 μg/ml for SuSa and SuSa-CP), there were four-fold fewer micronuclei in the resistant lines compared with the parent lines.

Cross-resistance

The results are the means of 3–5 experiments for each cell line and each drug. RT112-CP and SuSa-CP had different levels of resistance to cisplatin in the MTT assay compared with the colony-forming assay: RT112-CP ten-fold and SuSa-CP two-fold (Table 5). This finding was probably due to the different exposure time (6 days in the MTT assay, 1 h in the colony-forming assay), different population doubling-times (SuSa and SuSa-CP were significantly slower than RT112 and RT112-CP), and the fact that the MTT assay measures cytostasis as well as cytotoxicity. Similar levels of cross-resistance to carboplatin were observed. There was also a high degree of cross-resistance to the unrelated compound, methotrexate (RT112-CP seven-fold, SuSa-CP three-fold). Only to doxorubicin was there no cross-resistance.

DISCUSSION

To develop a model of acquired resistance to cisplatin, a testicular and a bladder tumour cell line were continuously exposed to the drug for 11 and 14 months. Characterisation of the resistant cell lines showed stable properties despite long-

Table 4. Micronucleus production 72 h after cisplatin exposure, mean (S.E.)

RT112		RT112-CP		SuSa		SuSa-CP	
CP	% MN	CP	% MN	CP	% MN	CP	% MN
2	2.0 (0.4)	8	1.3 (0.4)	0.5	3.4 (0.4)	2	1.8 (0.02)
4	3.1 (0.8)	16	2.9 (0.8)	1	5.3 (0.4)	4	2.9 (0.6)
8	5.3 (0.5)	32	4.1 (0.2)	2	8.3 (0.3)	8	4.6 (0.7)

CP = cisplatin concentration (μg/ml).

% MN = percentage of cells with micronuclei minus background. Background levels: RT112 0.8 (0.2); RT112-CP 1.8 (0.2); SuSa 4.8 (0.7); and SuSa-CP 3.4 (0.4).

Table 5. IC_{50}s of parent and resistant cell lines to therapeutic drugs (MTT assay)

	ng/ml		nmol/l		
	RT112	RT112-CP	RT112	RT112-CP	Ratio*
Bladder cell lines					
Cisplatin	64 (11)	638 (144)	213 (37)	2127 (480)	10.0
Carboplatin	1800 (400)	18,100 (2100)	4848 (1077)	48,748 (5656)	10.1
Methotrexate	1.1 (0.1)	7.3 (0.7)	2.4 (0.2)	16.1 (1.5)	6.6
Bleomycin	49 (11)	135 (29)	35 (7.8)	96 (21)	2.8
Etoposide	185 (20)	346 (57)	314 (34)	588 (97)	1.9
Doxorubicin	12.3 (0.9)	12.6 (0.2)	21.2 (1.5)	21.7 (0.3)	1.0
	ng/ml		nmol/l		
	SuSa	SuSa-CP	SuSa	SuSa-CP	Ratio
Testicular cell lines					
Cisplatin	41 (8)	87 (12)	137 (27)	290 (40)	2.1
Carboplatin	500 (55)	1000 (100)	1347 (148)	2693 (269)	2.0
Methotrexate	8.6 (0.6)	25.6 (7.1)	18.9 (1.3)	56.4 (5.6)	3.0
Bleomycin	6.8 (0.6)	7.9 (1.3)	4.9 (0.4)	5.6 (0.9)	1.2
Etoposide	11.3 (1.7)	20.5 (2.3)	19.2 (2.9)	34.8 (3.9)	1.8
Doxorubicin	1.7 (0.2)	1.6 (0.1)	2.9 (0.3)	2.8 (0.2)	1.0

IC_{50} = concentration causing 50% decrease in optical density compared with controls.
*Ratio IC_{50} of resistant line/IC_{50} of parent line.

term culture in cisplatin. Growth rates (population doubling-time and intermitotic time) were the same in parent and resistant cell lines. This result contrasts with that in several cisplatin-resistant cell lines in which doubling-time increased with induction of resistance [19–21]. The identical isozyme profiles of SuSa and SuSa-CP confirmed their common origin. The uncommon phenotype of GOTm seen in both RT112 and RT112-CP, and the identical patterns seen in these two cell lines with the hypervariable locus-specific probes λMS1 and pλg3, were conclusive evidence for their origin from the same individual. The changes seen in PGM1 and ESD could be due to the selection of a subpopulation of cells with a difference isozyme pattern during long-term culture in cisplatin. However, karyotyping did not provide support for this; there was no subpopulation in RT112 with gross chromosomal changes. One of the probes used for DNA analysis, λMS1, is derived from chromosome 1p, and the PGM1 locus is also on 1p. Since both RT112 and RT112-CP were heterozygous for λMS1, gross deletion of 1p did not account for the lack of expression of PGM1 in the parent line. However, karyotypic analysis showed that there was a deletion of 1p in RT112-CP in the region of the PGM1 locus (1p21–22). Rearrangement in this area may have altered expression of the PGM1 gene. It seems likely that PGM1 expression can occasionally 'switch off' in cultured cells, as in the erythroleukaemia line K562 [22]. Disappearance of the 6q+ marker, which is probably a 6/13 translocation, and appearance of the 13q− marker in RT112-CP may account for the change in ESD expression. There were no homogeneously staining regions or double minutes, and there was no marker common to RT112-CP and SuSa-CP that might account for their increased resistance.

Stable cisplatin resistance has been induced in human cell lines derived from other histological types of tumour, including head and neck [23], ovarian [24–27], and lung [20, 21] cancers and a Burkitt's lymphoma [23]. Mechanisms of resistance in these cell lines are multifactorial. The SCC-25/CP cell line (derived from a squamous cell carcinoma of the tongue) has decreased cisplatin uptake, increased protein sulphydryl (metallothionein) content, increased activity of an enzyme (glutathione-S-transferase) involved in glutathione metabolism, and decreased levels of DNA cross-linking. However, glutathione levels are unchanged [28]. Different mechanisms contribute to resistance in different cell lines. For example, within the ovarian cell lines, glutathione content is increased in 2780 CP [29], but is unchanged in 2008/DDP and COLO/DDP [24].

Initial studies on resistance in an RT112-CP subline (when cells were able to grow continuously in 2.5 μg/ml cisplatin) showed no difference in cisplatin uptake between RT112 and RT112-CP [30]. There were only minor differences in the amounts of cisplatin binding to DNA, and the peak levels of inter-strand crosslinking of DNA by cisplatin were similar. However, glutathione content was increased in this RT112-CP subline and there was increased activity of two enzymes involved in glutathione metabolism (glutathione reductase and glutathione peroxidase) which may, at least partly, explain the increased resistance [30].

The micronucleus assay has been used to study chromosomal damage caused by several anticancer agents, including cisplatin [31, 32]. Cisplatin is believed to exert its cytotoxic effect through interaction with DNA [33]. The drug causes chromosomal aberrations [34, 35] and mutations to 6-thioguanine resistance [36], but the precise nature of the cytotoxic lesion is not known. We used the micronucleus assay to investigate the extent of cisplatin-induced DNA damage in sensitive and resistant cell lines. A similar dose response between the lethal effects and the

induction of micronuclei may indicate that chromosomal damage is the main cause of death for cells treated with cisplatin [31]. However, despite a similar relation between micronucleus production, cytotoxicity and because of the practical limitations of the assay (low frequency of micronuclei relative to cytotoxicity and lack of information obtained about mechanisms), we do not believe that there is sufficient evidence for a causal link.

Patterns of cross-resistance were similar in RT112-CP and SuSa-CP. In common with other studies [20, 26], both lines were cross-resistant to carboplatin, to the same extent as to the inducer. They were also highly cross-resistant to methotrexate, a drug with no structural or functional similarity to cisplatin. A high level of cross-resistance to methotrexate was also seen in the squamous cell carcinoma cell line SCC-25/CP [19, 23]. Neither RT112-CP nor SuSa-CP were cross-resistant to doxorubicin which suggests a possible clinical use for doxorubicin as a second-line agent in patients resistant to cisplatin. Other cisplatin-resistant cell lines also lack cross-resistance or are more sensitive to doxorubicin [20, 23, 26].

1. Peckham M. Testicular cancer. *Rev Oncol* 1988, **1**, 439–453.
2. Sternberg CN, Yagoda A, Scher HI *et al.* M-VAC (methotrexate, vinblastine, doxorubicin and cisplatin) for advanced transitional cell carcinoma of the urothelium. *J Urol* 1988, **139**, 461–469.
3. Walker MC, Parris CN, Masters JRW. Differential sensitivities of human testicular and bladder tumor cell lines to chemotherapeutic drugs. *J Natl Cancer Inst* 1987, **79**, 213–216.
4. Parris CN, Arlett CF, Lehmann AR, Green MHL, Masters JRW. Differential sensitivities to gamma radiation of human bladder and testicular tumour cell lines. *Int J Radiat Biol* 1988, **53**, 599–608.
5. Masters JRW, Hepburn PJ, Walker L *et al.* Tissue culture model of transitional cell carcinoma: characterization of twenty-two human urothelial cell lines. *Cancer Res* 1986, **46**, 3630–3636.
6. Hogan B, Fellous M, Avner P, Jacob F. Isolation of a human teratoma cell line which expresses F9 antigen. *Nature* 1977, **270**, 515–518.
7. Groos E, Walker L, Masters JRW. Intravesical chemotherapy: studies on the relationship between pH and cytotoxicity. *Cancer* 1986, **58**, 1199–1203.
8. Riddle PN. *Time-lapse Cinemicroscopy*. London, Academic Press, 1979.
9. Gratzner HG. Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: a new reagent for detection of DNA replication. *Science* 1982, **218**, 474–475.
10. Gratzner HG, Young IT, Sher SE. An immunocytochemical approach to cell kinetics automation. *J Histochem Cytochem* 1979, **27**, 496–499.
11. Harris H, Hopkinson DA. *Handbook of Enzyme Electrophoresis in Human Genetics*. Amsterdam, North Holland, 1976.
12. Edwards YH, Parkar M, Povey S, West LF, Parrington JM, Solomon E. Human myosin heavy chain genes assigned to chromosome 17 using a human cDNA clone as probe. *Anal Hum Genet* 1985, **49**, 101–109.
13. Wong Z, Wilson V, Patel I, Povey S, Jeffreys AJ. Characterization of a panel of highly variable minisatellites cloned from human DNA. *Ann Hum Genet* 1987, **51**, 269–288.
14. Feinberg AP, Vogelstein B. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 1984, **137**, 266–267.
15. Masters JRW, Bedford P, Povey S, Franks LM. Identity of some human bladder cancer cell lines revisited using DNA fingerprinting. *Br J Cancer* 1988, **57**, 284–286.
16. Freshney RI. *Culture of Animal Cells: a Manual of Basic Technique*. New York, Alan R Liss, 1983.
17. Heddle JA, Raj AS, Krepinsky AB. The micronucleus assay. II. *In vitro*. In: Stich HF, San RHC, eds. *Short-term Tests for Chemical Carcinogens*. New York, Springer, 1981, 250–254.
18. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983, **65**, 55–63.
19. Rosowsky A, Wright JE, Cucchi CA *et al.* Collateral methotrexate resistance in cultured human head and neck carcinoma cells selected for resistance to *cis*-diamminedichloroplatinum(II). *Cancer Res* 1987, **47**, 5913–5918.
20. Hong WS, Saijo N, Sasaki Y *et al.* Establishment and characterization of cisplatin-resistant sublines of human lung cancer cell lines. *Int J Cancer* 1988, **41**, 462–467.
21. Hospers GAP, Mulder NH, de Jong B *et al.* Characterization of a human small cell lung carcinoma cell line with acquired resistance to *cis*-diamminedichloroplatinum(II) *in vitro*. *Cancer Res* 1988, **48**, 6803–6807.
22. Povey S, Jeremiah S, Arthur E, Steel M, Klein G. Differences in genetic stability between human cell lines from patients with and without lymphoreticular malignancy. *Ann Hum Genet* 1980, **44**, 119–133.
23. Teicher BA, Cucchi CA, Lee JB, Flatow JL, Rosowsky A, Frei E III. Alkylating agents: *in vitro* studies of cross-resistance patterns in human cell lines. *Cancer Res* 1986, **46**, 4379–4383.
24. Andrews PA, Murphy MP, Howell SB. Differential potentiation of alkylating and platinating agent cytotoxicity in human ovarian carcinoma cells by glutathione depletion. *Cancer Res* 1985, **45**, 6250–6253.
25. Kikuchi Y, Miyauchi M, Kizawa I, Oomori K, Kato K. Establishment of a cisplatin-resistant human ovarian cancer cell line. *J Natl Cancer Inst* 1986, **77**, 1181–1185.
26. Behrens BC, Hamilton TC, Masuda H *et al.* Characterization of a *cis*-diamminedichloroplatinum(II)-resistant human ovarian cancer cell line and its use in evaluation of platinum analogues. *Cancer Res* 1987, **47**, 414–418.
27. Kuppen PJK, Schuitemaker H, van't Veer LJ, de Bruijn EA, van Oosterom AT, Schrier PI. *cis*-Diamminedichloroplatinum(II)-resistant sublines derived from two human ovarian tumor cell lines. *Cancer Res* 1988, **48**, 3355–3359.
28. Teicher BA, Holden SA, Kelley MJ *et al.* Characterization of a human squamous carcinoma cell line resistant to *cis*-diamminedichloroplatinum(II). *Cancer Res* 1987, **47**, 388–393.
29. Hamilton TC, Winker MA, Louie KG *et al.* Augmentation of Adriamycin, melphalan and cisplatin cytotoxicity in drug-resistant and -sensitive human ovarian carcinoma cell lines by buthionine sulfoximine mediated glutathione depletion. *Biochem Pharmacol* 1985, **34**, 2583–2586.
30. Bedford P, Shellard SA, Walker MC, Whelan RDH, Masters JRW, Hill BT. Differential expression of collateral sensitivity or resistance to cisplatin in human bladder carcinoma cell lines pre-exposed *in vitro* either to X-irradiation or cisplatin. *Int J Cancer* 1987, **40**, 681–686.
31. Bonatti S, Lohman PHM, Berends F. Induction of micronuclei in Chinese hamster ovary cells treated with Pt co-ordination compounds. *Mutation Res* 1983, **116**, 149–154.
32. Kliesch U, Adler ID. Micronucleus test in bone marrow of mice treated with 1-nitropropane, 2-nitropropane and cisplatin. *Mutation Res* 1987, **192**, 181–184.
33. Roberts JJ, Thomson AJ. The mechanism of action of antitumor platinum compounds. *Prog Nucleic Acid Res Mol Biol* 1979, **22**, 71–133.
34. Van den Berg HW, Roberts JJ. Post-replication repair of DNA in Chinese hamster cells treated with *cis*-platinum(II) diammine dichloride. Enhancement of toxicity and chromosome damage by caffeine. *Mutation Res* 1975, **33**, 279–284.
35. Turnbull D, Popescu MC, DiPaolo JA, Myhr BC. *cis*-Platinumdiammine-dichloride causes mutation, transformation and sister-chromatid exchanges in cultured mammalian cells. *Mutation Res* 1979, **66**, 267–275.
36. Plooy ACM, Lohman PHM. Platinum compounds with antitumour activity. *Toxicology* 1979, **17**, 168–176.

Acknowledgements—We thank A. Kearney for the isozyme and DNA analyses, and L.F. West for the chromosome preparation and staining. The work was supported in part by a grant from the Nuffield Foundation.