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Acknowledgements——This work was supported by the Dr Mildred Scheel Stiftung W57/91/Re2. DFMO was a gift from Merrell Dow Company, Cincinnatti, Columbus, U.S.A. We express our appreciation to M. Ertz, P. Krück and I. Wagner-Gillen for their expert technical assistance. We also thank Dr T. Plant for critically reading this manuscript.

Pergamon

European Journal of Cancer Vol. 31A, No. 6, pp. 981–986, 1995

0959-8049/95 $9.50 + 0.00

0959-8049(95)00198-0

Relationships Between Resistance to Cisplatin and Antifolates in Sensitive and Resistant Tumour Cell Lines

L.R. Kelland, R. Kimbell, A. Hardcastle, G.W. Aherne and A.L. Jackman

Possible relationships between tumour resistance to cisplatin and the folate-based thymidylate synthase (TS) inhibitors, CB3717 and ZD1694 (tomudex), have been investigated *in vitro* using a panel of tumour cell lines (predominantly human ovarian), either parental or possessing acquired resistance to cisplatin or ZD1694. Across eight parent human tumour cell lines, ZD1694 was the most potent drug (mean IC_{50} of 1.9×10^{-8} M), being over 250 times as potent as its prototype CB3717 (mean IC_{50} of 4.8×10^{-6} M). In five pairs of acquired cisplatin-resistant human tumour cell lines (three ovarian, one cervical and one testicular) which encompass all of the main known mechanisms of platinum drug resistance, ZD1694, CB3717 and the DHFR inhibitor, methotrexate, all exhibited non-cross-resistance. The cervical line, HX/155cisR, showed collateral sensitivity to ZD1694, CB3717, 5-fluorouracil (FUra) and fluorodeoxyuridine (FdUrd). One cell line, A2780cisR, showed a low level of cross-resistance to FUra (resistance factor, RF, of 1.5) and FdUrd (RF of 3.8). A2780cisR, in common with two other cisplatin-resistant lines, did not possess elevated TS activity compared with its parent. Cisplatin retained activity in four acquired ZD1694-resistant cell lines (encompassing reduced folate transport, elevated TS and defective polyglutamation mechanisms of resistance). Furthermore, combinations of ZD1694 with each of the platinum-based drugs, cisplatin, carboplatin and the recently introduced orally administrable, JM216, all showed additive growth inhibitory effects by median effect analysis. These data suggest that the tumour inhibitory properties of the recently introduced highly potent TS inhibitor, ZD1694, and cisplatin, and, moreover, their respective mechanisms of resistance, do not overlap. Therefore, these drugs may be considered for combination in the clinic.

Key words: tomudex, platinum, antifolates, resistance
Eur J Cancer, Vol. 31A, No. 6, pp. 981–986, 1995

INTRODUCTION

EFFORTS TO overcome the clinical problem of tumour drug resistance commonly involve the usage of combinations of drugs with distinct modes of action, non-overlapping mechanisms of acquiring resistance and, preferably, non-overlapping dose-limiting toxicities. For human ovarian cancer, the platinum-based drugs cisplatin and carboplatin are now generally accepted as being the most active single agents, producing complete responses in at least 50% of patients presenting with advanced disease [1]. However, the majority of tumours from these responding patients eventually develop resistance to cisplatin resulting in disappointing long-term survival rates [1]. While Taxol (paclitaxel) has recently been shown to offer some activity (about 25% response rate) in platinum-refractory disease [2] there remains scope for considerable improvement.

This study has attempted to address whether a clinical role might exist for combining a platinum-based drug with potent inhibitors of thymidylate synthase (TS, EC 2.1.1.45). Some supportive evidence for such a role arose from the phase I/II clinical trials of the prototype folate-based TS inhibitor, CB3717 (N^{10}-propargyl-5,8-dideazafolic acid) where, despite the curtailment of the trial due to severe dose-limiting nephrotoxicity, some evidence of responsiveness was observed in platinum-refractory ovarian cancer [3]. In addition, *in vitro* studies have reported collateral sensitivity of cisplatin-resistant human non-small cell lung and colon cancer cell lines to the TS inhibitors 5-fluorouracil (FUra) and 5-fluoro-2'-deoxyuridine (FdUrd) [4, 5]. However, other studies have shown that cisplatin resistance is associated with an increase in TS enzyme activity [6–10] and, therefore, indicative of cross-resistance between platinum and TS inhibitors. To address this issue, we have used tumour (primarily human ovarian) cell lines and sublines selected for resistance to either cisplatin or ZD1694 [*N*-(5-[*N*-(3,4-dihydro-2-methyl-4-oxoquinazolin-6-yl-methyl)-*N*-methylamino]-2-then-oyl)-L-glutamic acid], tomudex, a new more potent and water soluble TS inhibitor than CB3717, which is currently undergoing phase II/III clinical trials, including in ovarian cancer [11–13]. Comparative growth inhibitory properties of CB3717, ZD1694 and cisplatin as well as FdUrd, FUra and the dihydrofolate reductase (DHFR) inhibitor methotrexate (MTX) have been performed in parallel with determinations of TS enzyme activity in cisplatin- and ZD1694-sensitive and -resistant cell lines.

MATERIALS AND METHODS

Cell lines

A total of 10 "parent" tumour cell lines have been used: SKOV-3, HX/62, PXN/94, 41M, CH1 and A2780 human ovarian carcinomas; HX/155, human cervical carcinoma; GCT27, human testicular teratoma; W1L2, human lymphoblastoid; and L1210, murine leukaemia. Biological details of each of these lines have been described previously [14–18]. With five of the above parent lines, sublines with acquired resistance to cisplatin have been established as described previously: 41McisR and CH1cisR [19]; HX/155cisR [16]; and GCT27cisR [17]. The A2780/A2780cisR pair of lines [15] were kindly provided by Dr T. Hamilton (Fox Chase Cancer Center, Philadelphia, Pennsylvania, U.S.A.).

In addition, sublines with derived resistance to ZD1694 have been used. Details of the derivation of these lines (41M ZD1694R, CH1 ZD1694R, W1L2 ZD1694R and L1210 ZD1694R) has been described previously [20]. Briefly, lines were made resistant by continual exposure to incremental doses of the drug. An increase in TS activity is the primary mechanism of resistance to ZD1694 in the W1L2- and CH1-resistant lines. In contrast, resistance in the 41MZD1694R and L1210ZD1694R lines is attributable to impaired drug transport and reduced polyglutamation, respectively. In addition, two L1210-derived sublines, L1210:1565 (resistant to antifolates which enter cells via the reduced folate carrier) and L1210:R7A (resistant to MTX through elevated levels of DHFR) and one W1L2-derived subline, W1L2:C1 (possesses acquired resistance to the TS inhibitor 2-desamino-2-methyl-N^{10}-propargyl-5,8-dideazafolate and overexpresses TS ~ 200-fold) have been used. Biological properties of these sublines have been summarised previously [18].

All lines with the exception of W1L2- and L1210-derived lines grew as monolayers in Dulbecco's modified Eagle's medium plus 10% fetal calf serum (undialysed), 50 μg/ml gentamicin, 2.5 μg/ml amphotericin B, 2 mM glutamine, 10 μg/ml insulin and 0.5 μg/ml hydrocortisone in a 10% CO_2, 90% air atmosphere. The W1L2 and L1210 lines grew as suspension cultures in RPMI 1640 medium without $NaHCO_3$, but containing 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid and supplemented with 10% horse serum (for L1210) or 10% fetal calf serum (for W1L2) (again undialysed). All cell lines were routinely checked throughout the course of these studies and found to be negative for the presence of *Mycoplasma* (Mycotect kit, Life Technologies Ltd, Paisley, Scotland).

Drugs

The platinum drugs, cisplatin [*cis*-diammine-dichloro-platinum(II)], carboplatin [*cis*-diammine-1,1-cyclobutane dicarboxylatoplatinum(II)] and JM216 [bis-acetato ammine cyclohexylamine dichloro platinum (IV)] were synthesised by and obtained from the Johnson Matthey Technology Centre (Reading, Berkshire, U.K.). The quinazoline antifolates CB3717 and ZD1694 were synthesised at Zeneca Pharmaceuticals (Alderley Park, Macclesfield, Cheshire, U.K.). MTX and FUra were obtained from David Bull Laboratories (Warwick, U.K.) and FdUrd from Sigma Chemicals (Poole, U.K.).

Assessment of growth inhibition

For the W1L2 and L1210 lines, cells were set up at 5×10^4/ml in 25-cm^2 tissue culture flasks and drugs added for a period of either 48 h (for L1210 lines) or 72 h (for W1L2) lines as previously described [11, 18, 20]. Thereafter, cell number counts were determined using a model ZM Coulter Counter.

For the remaining monolayer cultures, single cells (harvested after trypsinisation) were seeded at between 5×10^3 and 1×10^4 cells/well into 96-well microtitre plates. After overnight incubation to allow attachment, drugs were added at various concentrations in quadruplicate wells for a period of 96 h. Growth inhibition was then assessed as described previously [14] by adding L-4,5-^{3}H-leucine 16.7 μCi/ml (specific activity 130 Ci/mmol) for 180 min at 37°C. Cells were then trypsinised and harvested onto filters using a cell harvester (Inotech, Biosystems International Inc., Wohlen, Switzerland) and radioactivity per filter assessed using an automatic filter counting system (Inotech).

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Revised 21 Nov. 1994; accepted 1 Dec. 1994.

For both methods, the IC_{50} was defined as the concentration of compound required to inhibit cell growth by 50%.

Median effect analysis

Combinations of ZD1694 with either cisplatin, carboplatin or JM216 in constant molar ratios (determined from respective IC_{50} values from the growth inhibition curves for drug alone) were analysed by median effect analysis as described previously [21, 22]. Briefly, the analysis compares the effects of drug combinations to the effects of individual drugs across the entire dose–effect range. All drugs were added continuously for 96 h. Growth inhibition data were then fitted to regression lines, and the concentration of each drug which produced a given level of growth inhibition (fractional effect, Fa) alone, or in combination, was determined. Combination index (CI) for a given Fa (typically 0.5) was calculated as:

$$CI = \frac{d^1}{D_1} + \frac{d^2}{D_2}$$

where D_1 and D_2 are the doses of drugs one and two, which by themselves produce a given Fa (i.e. IC_{50}); d^1 and d^2 are the doses which produce the same Fa in combination. CI = 1 indicates zero interaction (additive cytotoxicity), CI < 1 indicates synergy and CI > 1 indicates antagonism (Chou computer program, Biosoft, Cambridge, U.K.).

Measurement of TS enzyme activity and levels

TS enzyme activity was assessed by ^3H release after a 1-h incubation of cytosols of whole cells (approximately 1×10^7 cells harvested in exponential phase) as described previously [11, 18, 20]. In addition, TS levels in parent and acquired cisplatin-resistant cell lines were determined by a competitive coated antigen ELISA using rabbit antibodies to recombinant human TS as described previously [23]. Briefly, approximately 5×10^6–1×10^7 cells were harvested by trypsinisation and the resulting cell pellet resuspended in 1 ml ice-cold phosphate-buffered saline containing 1% gelatin. Cells were then disrupted by freeze–thawing, vortexing and sonication, centrifuged (2500 rpm, 10 min, 4°C) and the resulting supernatant assayed by ELISA ([23] and Aherne *et al.*, manuscript in preparation). For measurements of both TS enzyme activity and levels, care was taken to ensure that cells were harvested in exponential growth (approximately 80% confluence).

Statistical analysis

Where indicated, statistical significance was tested using a two-tailed Student's *t*-test; a P value <0.05 was considered significant.

RESULTS

Initially, the comparative growth inhibitory effects of ZD1694, CB3717, FUra, FdUrd and MTX were determined against eight parent human tumour cell lines, five ovarian, one cervical and one testicular (Figure 1). ZD1694 was the most potent agent against the majority of the cell lines (mean IC_{50} across the eight lines of 1.9×10^{-8} M) and, overall, was over 250 times more potent than CB3717 (mean of 4.8×10^{-6} M). FdUrd (mean IC_{50} of 4.2×10^{-8} M) was around 200-fold more potent than FUra (mean IC_{50} of 8.1×10^{-6} M). MTX exhibited cytotoxicity broadly comparable to that of FdUrd (mean IC_{50} of 7×10^{-8} M).

The growth inhibitory effect of ZD1694, CB3717, FUra, FdUrd and MTX has been determined in five pairs of parent and acquired cisplatin-resistant human tumour cell lines (Table 1). These lines have been shown previously to encompass all of the known mechanisms of acquired resistance to cisplatin: reduced transport (the main mechanism of resistance in 41McisR and contributing to resistance in HX/155cisR and A2780cisR), increased glutathione levels (contributing to resistance in A2780cisR and HX/155cisR) and increased removal of platinum–DNA adducts (the main mechanism of resistance in CH1cisR and GCTcisR and contributing to resistance in A2780cisR) [15–17, 19]. Whereas all five cell lines exhibited resistance to cisplatin (ranging from 4.7- to 15.7-fold), non-cross-resistance (resistance factor, RF <1.5) was observed with ZD1694, CB3717 and MTX. One cell line, however (A2780cisR), showed a low level of cross-resistance to FUra (RF of 1.5) and partial cross-resistance to FdUrd (RF of 3.8). The cisplatin-resistant cervical cell line HX155cisR showed evidence of collateral sensitivity (RF <0.5) to ZD1694, CB3717, FUra and FdUrd. In addition, 41McisR showed evidence of collateral sensitivity to FUra and FdUrd.

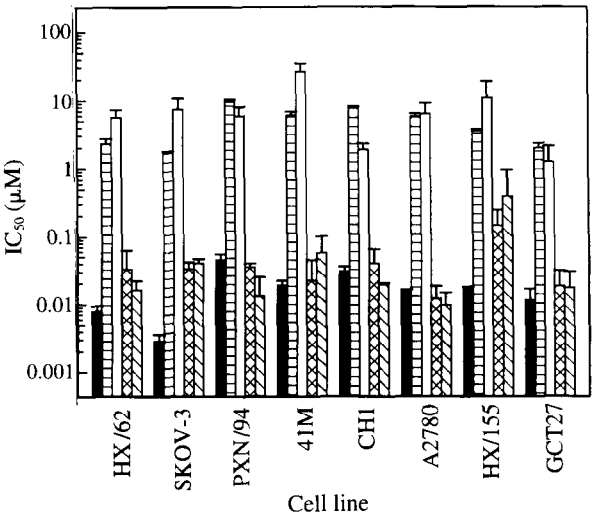

Figure 1. Growth inhibitory effects of ZD1694 (solid bars), CB3717 (horizontal hatched bars), 5-fluorouracil (open bars), fluorodeoxyuridine (cross-hatched bars) and methotrexate (diagonal hatched bars) against eight "parent" human tumour cell lines: HX/62, SKOV-3, PXN/94, 41M, CH1 and A2780 ovarian; HX/155 cervical; and GCT27 testicular. Values are mean ± S.D., n = 3–6.

The activity of cisplatin against four pairs of parent and acquired D1694-resistant cell lines is shown in Table 2. As for the acquired cisplatin-resistant lines, these lines have been shown to encompass a variety of mechanisms of resistance to ZD1694 (Table 2). However, in lines ranging from 20- to 8000-fold resistance to ZD1694 and regardless of the mechanism of resistance, cisplatin retained activity in all four lines (RF <1.5). Some evidence of collateral sensitivity was apparent in L1210ZD1694R (RF of 0.55). In addition, non-cross-resistance to carboplatin was observed in the W1L2ZD1694R and L1210ZD1694R lines; mean IC_{50} values (μM), W1L2 6.5 ± 0.6, W1L2ZD1694R 9.2 ± 1.5 (RF 1.4), L1210 12.2 ± 0.99, L1210ZD1694R 7.2 ± 0.8 (RF 0.59). Cisplatin and carboplatin also exhibited non-cross-resistance in three additional cell lines: W1L2C1, a TS overproducer [18] (RF values of 1 ± 0.18 for cisplatin and 0.99 ± 0.1 for carboplatin); L12101565, a reduced folate carrier defective line [18] (RF values of 0.72 for cisplatin

Table 1. Growth inhibitory effects against acquired cisplatin-resistant cell lines

Cell line pair	Resistance factor					
	Cisplatin	ZD1694	CB3717	FUra	FdUrd	MTX
41M/41McisR	4.7	1.4 ± 0.13	0.92 ± 0.06	0.56	0.56	0.82
CH1/CH1cisR	6.4	1.0 ± 0.075	1.02 ± 0.015	1.2	0.94	1.1
A2780/A2780cisR	15.7	1.0 ± 0.11	0.73 ± 0.04	1.5	3.8	1.1
HX155/HX155cisR	8.5	0.37 ± 0.08	0.23 ± 0.01	0.21	0.43	0.9
GCT27/GCTcisR	5.5	0.9 ± 0.3	0.88 ± 0.07	0.87	1.2	1.1

Resistance factor: IC_{50} resistant/IC_{50} parent line. Values are mean ± S.D. where indicated; n = 3–6. FUra, 5-fluorouracil; FdUrd, fluorodeoxyuridine; MTX, methotrexate.

Table 2. Growth inhibitory effects of cisplatin and carboplatin against acquired ZD1694-resistant cell lines

Cell line pair	Major mechanism of resistance	Resistance factor		
		ZD1694	Cisplatin	Carboplatin
41M/41MZD1694R	Reduced transport	94	1.4 ± 0.28	ND
CH1/CH1ZD1694R	Increased TS activity	20	1.1 ± 0.4	ND
W1L2/W1L2ZD1694R	Increased TS activity	65	1.35 ± 0.15	1.41 ± 0.01
L1210/L1210ZD1694R	Polyglutamation defect	8000	0.55 ± 0.09	0.59 ± 0.014

Resistance factor: IC_{50} resistant/IC_{50} parent line. Values are mean ± S.D. where indicated; n = 3–6. ND, not determined; TS, thymidylate synthase.

and 0.65 for carboplatin); and L12107RA, a DHFR overproducer [18] (RF values of 1.43 for cisplatin and 0.53 for carboplatin).

Combinations of ZD1694 with the platinum-based drugs, cisplatin, carboplatin and JM216, have been used in the CH1 and 41M cells and their acquired cisplatin-resistant sublines and growth inhibitory effects analysed by median effect analysis (Table 3). JM216 was included since it has recently entered clinical trials as the first orally administrable platinum drug [24]. Results showed essentially additive effects (CI values at Fa = 0.5 not significantly different from 1; $P > 0.05$) for both cell lines for all three drug combinations.

TS enzyme activity has been determined in three pairs of parent and acquired cisplatin-resistant cell lines (41M, CH1 and A2780). None of the acquired cisplatin-resistant cell lines showed a significant difference in TS enzyme activity from that of its respective parent line ($P > 0.05$). TS activity (nmol product/10^6 cells/h) values (means ± S.D., n = 3) were as follows: 41M 2.0 ± 0.4, 41McisR 2.7 ± 1 (difference 1.4-fold); CH1 3.3 ± 0.2, CH1cisR 2.5 ± 0.4 (difference 0.8-fold); A2780 4.4 ± 1.0, A2780cisR 4.0 ± 0.6 (difference 0.9-fold). In addition, TS levels have been determined in parent and acquired cisplatin-resistant lines by ELISA. While there was no clear difference in levels between resistant and parent counterparts

Table 3. Median effect analysis of the interaction between the platinum-based drugs, cisplatin, carboplatin and JM216 and ZD1694 in CH1/CH1cisR and 41M/41McisR human ovarian carcinoma cell lines

Drug pair	Combination index at Fa = 0.5			
	CH1	CH1cisR	41M	41McisR
ZD1694 and cisplatin (1:20 ratio)	1.35 ± 0.44	0.78 ± 0.26	1.4	1.1
ZD1694 and carboplatin (1:200 ratio)	0.97 ± 0.1	0.92 ± 0.13	1.1	1.3
ZD1694 and JM216 (1:10 ratio)	1.1 ± 0.2	1.2 ± 0.22	ND	ND

Values are mean ± S.D. (n = 3) for CH1/CH1cisR lines. Values for 41M and 41McisR are from a single determination. ND, not determined; Fa, fractional effect.

for four of the pairs, A2780cisR contained 1.6-fold greater levels than A2780 (n = 4, mean ± S.D. 639 ± 286 versus 404 ± 286 fmoles/10^6 cells, respectively), although this difference did not reach statistical significance (P = 0.2).

DISCUSSION

Since the demonstration of clinical activity with CB3717 (including in patients with ovarian cancer) [3], TS has been advocated as a particularly promising target enzyme for anticancer drug design. This study, using eight parent human tumour cell lines (mainly ovarian) has embellished our previous findings using murine L1210 leukaemia cells of the enhanced potency of the new water-soluble folate-based TS inhibitor ZD1694 over its prototype CB3717 [11]. ZD1694 was on average, of over 250 times more potent than CB3717 and, in addition, was an average of 200-fold more potent than FUra, 2.2-fold more potent than FdUrd and 3.7-fold more potent than MTX. Interestingly, the two lines most sensitive to the growth inhibitory effects of ZD1694, HX/62 and SKOV-3 have previously been reported to be the most resistant of this panel to cisplatin (and other bifunctional alkylating agents such as chlorambucil) [14]. ZD1694 has also exhibited promising *in vivo* activity against the corresponding HX/62 xenograft counterpart [25]. Although ZD1694 is 20-fold less effective as an inhibitor of isolated TS than CB3717 [11], as alluded to previously, the enhanced potency of ZD1694 over CB3717 is likely to be due to its superior substrate activity for folylpolyglutamate synthetase (FPGS), leading to the much more rapid formation of polyglutamates (mainly ranging from the tri- to penta forms) [26]. The polyglutamates of ZD1694 have been shown to be retained within cells (including in the 41M and CH1 lines used in this study) and, for example, the pentaglutamate was over 100-fold more potent as an inhibitor of isolated TS than the parent monoglutamate [11, 26].

ZD1694 (tomudex) is now in phase II/III clinical trials, including in patients with platinum refractory ovarian cancer [12, 13]. As some studies (notably those of Scanlon and co-workers [6–8]) have suggested that cisplatin resistance is associated with an increase in TS activity (and thus TS inhibitors might be expected to exhibit general cross-resistance with platinum-based drugs), this *in vitro*-based study has also explored the feasibility, in terms of tumour drug resistance, of combining ZD1694 with the platinum-based drugs, cisplatin or carboplatin, widely recognised as the most clinically useful agents for the chemotherapy of ovarian cancer [1]. Using five pairs (parent and subline with acquired cisplatin resistance) of human tumour cell lines (three ovarian, one cervical, one testicular), our results demonstrate a lack of cross-resistance between cisplatin and ZD1694. Indeed, evidence of collateral sensitivity was apparent for one line (HX/155cisR). Moreover, in four pairs of lines selected for resistance to ZD1694, non-cross-resistance to cisplatin was observed. Two ovarian cell lines, 41M and CH1, were common to both panels of resistant lines. Furthermore, parallel mechanistic studies have shown that, within both panels, all of the main mechanisms of resistance to both cisplatin (reduced drug uptake, enhanced intracellular detoxification through elevated glutathione and increased removal of platinum–DNA adducts) and ZD1694 (reduced folate transport, increased TS levels and defective polyglutamation) have been encompassed [15–20].

The published literature relating to platinum drug resistance and TS is equivocal, probably reflecting differences in the individual phenotypic properties of each selected resistant line. For example, at least two other studies using human non-small cell lung cancer [4] and the colon BE cancer [5] acquired cisplatin resistant lines have shown, in common with our HX/155cisR cervical carcinoma cell line, collateral sensitivity between TS inhibitors (FUra, and FdUrd in the lung study) and cisplatin. However, it should be noted that, as well as inhibitory effects on TS through production of 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP), FUra has also been shown to exert additional effects directly on RNA which probably also contribute to its antitumour effects [27]. At present, the mechanism(s) underlying this observation is/are unclear and worthy of further study. In common with the HX/155cisR and HX/155 pair of lines, no difference in TS activity was observed within the pairs of acquired cisplatin-resistant human non-small cell lung lines [4]. In a subsequent study, the collateral sensitivity to FUra was associated with an inhibition of dTTP synthesis, probably mediated through decreased uptake of (thymidine) dThd in the cisplatin-resistant line [28].

In contrast to our data and the above reports, other studies have shown cross-resistance between cisplatin and TS inhibitors (again usually using FUra) correlating to an increased activity of TS in acquired cisplatin-resistant cell lines [6–10]. To date, no other study has reported data using ZD1694. Notably, Scanlon and co-workers also used the A2780/A2780cisR pair of lines for their studies and reported a 3-fold increase in mRNA and a 2.5-fold increase in enzyme activity for both TS and DHFR in the resistant compared to the sensitive line [6]. This was reflected in cross-resistance to FUra (3.2-fold) and MTX (2.8-fold); the line was 3.2-fold resistant to cisplatin [7]. Intriguingly, while A2780cisR in our hands also showed partial cross-resistance to FdUrd (3.8-fold) and a low level of cross-resistance to FUra (1.5-fold), no cross-resistance was observed to either ZD1694, CB3717 or MTX [TS levels were also slightly, but not significantly, higher (1.6-fold) but TS activity was similar in both lines]. This interesting observation is, as yet, unexplained and merits further investigation including determination of the relative roles of apoptotic pathways and dTMP pools in the pair of cell lines. In a further study using HCT8/HCT8cisR colon carcinoma cells, Scanlon and colleagues showed that cisplatin resistance was again associated with a 4.2-fold increase in TS enzyme activity and cross-resistance to FUra, FdUrd and MTX [8]. Based on their studies, Scanlon and colleagues have proposed that the TS cycle provides the sole source of *de novo* thymidylate required by various repair enzymes involved in removing platinum–DNA adducts [29]. However, in our acquired cisplatin-resistant cell lines where enhanced DNA repair has been shown to play a role in determining resistance (CH1cisR and GCT27cisR [19, 17]), no differences in TS levels and TS activity and a lack of cross-resistance to TS inhibitors was observed. Furthermore, in two ZD1694-resistant lines (CH1ZD1694R and W1L2ZD1694R) where increased TS activity and increased TS levels were observed [20], no obvious cross-resistance to cisplatin was apparent.

As for FUra, cisplatin-resistant cell lines have been described showing either cross-resistance [7–9, 30] or collateral sensitivity [5, 10] to MTX. Mechanistic studies have ascribed cross-resistance in SCC25 human squamous carcinoma of the head and neck cells to the reduction in a membrane protein recognised by monoclonal antibody SQM1 [31]. Collateral sensitivity in TR170 human ovarian carcinoma cells was associated with increased drug uptake [10].

In summary, our results demonstrate a lack of cross-resistance between cisplatin and ZD1694 in a number of human tumour

cell lines and additive growth inhibitory effects when used in combination. In addition, in the phase I trials of tomudex, objective responses were observed in refractory ovarian cancer [12]. However, it should be noted that in the initial phase II trial of single-agent tomudex in heavily pretreated platinum-resistant ovarian cancer (31 patients) only 2 patients had a partial remission (6.5% objective response rate) [13]. Nevertheless, as the dose-limiting toxicities of ZD1694 (gastrointestinal toxicity, fatigue and myelosuppression was dose-limiting in the initial phase I trial [12]) and cisplatin (and carboplatin) are generally non-overlapping, these findings suggest that ZD1694 may be usefully combined with either cisplatin or carboplatin in the clinical setting.

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Acknowledgements—This work was supported by grants to the Institute of Cancer Research: Royal Cancer Hospital from the Cancer Research Campaign and the Medical Research Council. We thank Melody Brown and the late George Abel for technical assistance.