Cancer Chemother Pharmacol (1992) 30: 43–50

Cancer Chemotherapy and Pharmacology

Human ovarian-carcinoma cell lines and companion xenografts: a disease-oriented approach to new platinum anticancer drug discovery*

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Received 24 September 1991/Accepted 7 January 1992

Summary. A disease-oriented approach to the discovery of novel platinum anticancer drugs has been established through the setting up of parallel human ovarian-carcinoma cell lines and xenografts. The correlation between in vitro and in vivo antitumour activity was determined for four reference platinum agents (cisplatin, carboplatin, iproplatin and tetraplatin) in eight companion lines. Two methods of assessing antitumour effect were used in vitro (tritiated thymidine incorporation and sulforhodamine B staining) and three were applied in vivo [28-day treated/control (T/C) ratio, growth delay and specific growth delay]. In vitro, large differences in cytotoxicity across the cell lines were observed for each drug. This was also reflected in the xenografts for cisplatin and carboplatin and, to a lesser extent, for iproplatin. A correlation analysis of in vitro vs in vivo data revealed a high, statistically significant positive correlation for cisplatin and a strong positive correlation for carboplatin. However, for the two platinum(IV) drugs, the correlation was less good. In particular, tetraplatin was markedly less active in vivo (showing a general lack of activity against all of the tumour lines) than its in vitro potency against the cell lines predicted, resulting in poor correlation coefficients. These human tumour panels may be valuable for the elucidation of both cellular/molecular and corresponding in vivo pharmacological mechanisms of platinum drug resistance. Moreover, the HX/62 and SKOV-3 tumour lines, which exhibit a level of intrinsic resistance to the four reference agents both in vitro and in vivo (and which were derived from patients who had not received prior platinum therapy), represent particularly useful evaluation models for the discovery of novel broad-spectrum platinum drugs.

* This study was supported by grants to the Institute of Cancer Research from the Cancer Research Campaign and the Medical Research Council, the Johnson Matthey Technology Centre and Bristol Myers Squibb Oncology

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Introduction

The traditional approach to the preclinical antitumour evaluation of potential anticancer drugs has generally involved the use of rapidly growing transplantable murine tumour lines [14, 18, 39]. Predominant among these in platinum drug development to date have been the L1210 and P388 leukaemias and, particularly in our previous studies leading to the discovery of carboplatin and iproplatin, the ADJ/PC6 plasmacytoma [20]. Subsequently, an extension of these models was provided through the derivation of variants showing acquired resistance to cisplatin. This led to the discovery that platinum complexes possessing a diaminocyclohexane [DACH] ligand retain activity against L1210 cell lines exhibiting derived resistance to cisplatin [5–7]. However, it is clear from our studies that although the DACH-containing complex tetraplatin [*trans*-d,l-1,2-diaminocyclohexanetetrachloroplatinum(IV); Ormaplatin, NSC 363812] retained activity against an L1210 showing acquired resistance to cisplatin, it failed to display activity against a correspondingly resistant murine ADJ/PC6 line [21]. To date, the clinical relevance of the DACH carrier ligand has largely remained untested, as previous phase I/II trials of JM82 [DACCP, 1,2-diaminocyclohexane(4-carboxyphthalato)platinum(II)] and the closely related TNO-6 [diaminomethylcyclohexane platinum(II) sulfate] were dropped, primarily due to unacceptable toxicity [10, 26, 35]. At present, three additional DACH-containing platinum drugs are undergoing early clinical evaluation: tetraplatin [1, 9], oxaliplatin [1,2-diaminocyclohexane-oxalato platinum(II)] [12] and the liposome-entrapped L-NDDP (*cis*-bis-neodecanoato-*trans*-*R*,*R*-1,2-diaminocyclohexaneplatinum(II) ([8]; see [28] for a review).

Our current efforts are aimed at the discovery of a new generation of platinum anticancer drugs that are capable of circumventing the problems of both intrinsic and acquired resistance to cisplatin/carboplatin. The existence of model inconsistencies such as those described above highlights the necessity of using models that are more predictive of clinical response. This has involved a general move in recent years away from rapidly growing murine tumours to

human tumour panels that conceptually might be more predictive of the target disease in man. This has culminated in a switch of the entire anticancer drug-screening programme at the National Cancer Institute (NCI) to multiple panels of in vitro human tumour cell lines [4]. For some tumour types, companion in vivo xenograft lines have been established.

As a step in achieving our objectives, we have established and described in vitro [24] and in vivo [22] laboratory models of human ovarian carcinoma, a disease in which both intrinsic and acquired resistance to currently available platinum-based chemotherapy severely limits a successful clinical outcome. Six of these tumour models are common to both the in vitro and the in vivo panels; two additional in vitro lines and their in vivo counterparts were established in the present investigations.

The aim of this study was to explore whether the predictive properties afforded by the in vitro cell lines would be reflected in the parallel in vivo panel. We determined the relationship between the antitumour effects produced by four reference platinum drugs (cisplatin, carboplatin, iproplatin and tetraplatin) in the in vitro panel vs the eight companion xenograft lines. Two independent methods of assessment were used in vitro (tritiated thymidine incorporation and sulforhodamine B staining) and three were applied in vivo (28-day treated/control ratios, growth delay and specific growth delay).

Materials and methods

Platinum agents

The platinum-containing agents cisplatin [CDDP, Neoplatin, *cis*-diamminedichloroplatinum(II)], carboplatin [JM8, CBDCA, Paraplatin, *cis*-diammine-1,1-cyclobutanedicarboxylatoplatinum(II)] and iproplatin [JM9, CHIP, *cis*-dichloro-*trans*-dihydroxo-*cis*-bis(isopropylamine)platinum(IV)] were synthesised by and obtained from the Johnson Matthey Technology Centre (Reading, Berkshire, UK). Tetraplatin was kindly provided by Dr. M. Wolpert-Defilippes (NCI, Bethesda, Md., USA).

In vitro lines

Cell lines. Eight human ovarian-carcinoma cell lines were used. SKOV-3 [13] and OVCAR-3 [19] were obtained from the American Type Culture Collection. Establishment details and biological properties of the other six cell lines (HX/62, PXN/94, CH1, LK1, LK2 and 41M) have been described elsewhere [24, 29]. All eight cell lines grew as monolayers in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated (55° C, 30 min) foetal calf serum (Imperial Laboratories, Andover, UK), 50 μg gentamicin/ml, 2.5 μg amphotericin B/ml, 2 mM L-glutamine, 10 μg insulin/ml and 0.5 μg hydrocortisone/ml in 10% CO_2/90% air. Cells were free of mycoplasma contamination throughout the course of the study.

Assessment of cytotoxicity. Immediately before their use, platinum agents were dissolved in 0.9% saline (cisplatin, iproplatin and tetraplatin) or water (carboplatin). Two independent methods of assessing cytotoxicity were applied. In all cases, cell lines were used within a defined range of 20 passages; during this period, no difference in population-doubling time or morphological appearance was apparent for any of the lines.

The tritiated thymidine incorporation assay was performed essentially as described elsewhere [24, 25]. Single viable cells were seeded at between 5×10^3 and 1×10^4 cells/well in 96-well microtitre plates in 200 μl growth medium. Agents were added on the following day to triplicate wells for a total exposure period of 96 h. [methyl-^{3}H]-Thymidine (4.2 μCi/ml sp. act., 5 Ci/mmol) was then added to each well for 60 min and cytotoxicity was assessed as previously described [24, 25].

In the sulforhodamine B (SRB) assay, cytotoxicity was assessed as described for the thymidine incorporation assay except that at the end of the 96-h exposure period, basic amino acid content was analysed using 0.4% SRB in 1% acetic acid (Sigma). The staining protocol has been described elsewhere [29].

All results represente mean values for at least three independent experiments. As demonstrated previously, the reproducibility of these in vitro assay systems is good, typically producing IC_{50} values (concentrations inhibiting the growth of 50% of the cell population) showing a maximal variation of ±25% after three determinations [24, 25, 29].

Table 1. Derivation of xenograft lines and doubling times for in vivo and in vitro lines

Line	Derivation	Doubling time	
		Xenograft (days)	Cell line (h)
HX/62	Solid biopsy	5.5	32
SKOV-3	Cell line	6.9	19
OVCAR-3	Cell line	14	35
PA1	Cell line	2.0	36
PXN/109T/C	Cell line (CH1)	8.4	17 (CH1)
PXN/94	Solid biopsy	15.2	23
LK2	Cell line	9.5	18
LK1	Cell line	9.8	20

In vivo lines

Cell lines. The derivation of the corresponding eight xenograft lines is shown in Table 1; lines were established either directly from the in vitro cell line or independently from biopsy material. The corresponding xenograft for the CH1 cell line was designated PXN/109T/C. In addition, Table 1 shows the doubling times determined for both the in vitro and the in vivo pairs of lines. All implants were carried out s. c. in anaesthetised female nude (nu/nu) mice (age, 6–8 weeks) using either 2-mm^3 biopsy fragments or 0.2 ml cell suspension containing 5×10^6 cells. Animals were housed in negative-pressure flexible film isolators and were maintained on a Labsure 21% protein diet (irradiated with 2.5 Mrad) with access to autoclaved tapwater ad libitum. Chemosensitivity testing was performed on tumours after passage 3, whereafter growth rates and histologies were stable.

Assessment of chemosensitivity. Platinum drugs were dissolved as described for the in vitro experiments and were injected i.p. at the previously determined maximum tolerated dose (MTD) for each agent (8 mg/kg for cisplatin, 100 mg/kg for carboplatin, 60 mg/kg for iproplatin and 8 mg/kg for tetraplatin; see [20–22]). As in our previous approach to the evaluation of platinum complexes [20–23], injections were performed on day 0 and, if well tolerated, every 7th day thereafter until day 21.

Mice bearing comparably sized tumours (diameter, approx. 8 mm) were randomised into treatment groups (n = 6 animals) or control groups (n = 10 animals). Tumour diameters (a, longest diameter; b, longest diameter at right angles to diameter a) were measured with a slide caliper every 7 days, and tumour volumes (V) were calculated according to the equation $V = a \times b^2 \times \pi/6$ and normalised to the value found at the start of treatment (day 0). Experiments were analysed by two methods: (1) 28-day T/C, or the ratio of the mean relative tumour volume of treated groups to that of control groups on day 28 post-treatment, and (2) growth delay, or the difference in the time required for control and treated tumours to double in volume. These parameters have been extensively used in our previous in vivo evaluation of platinum-containing com-

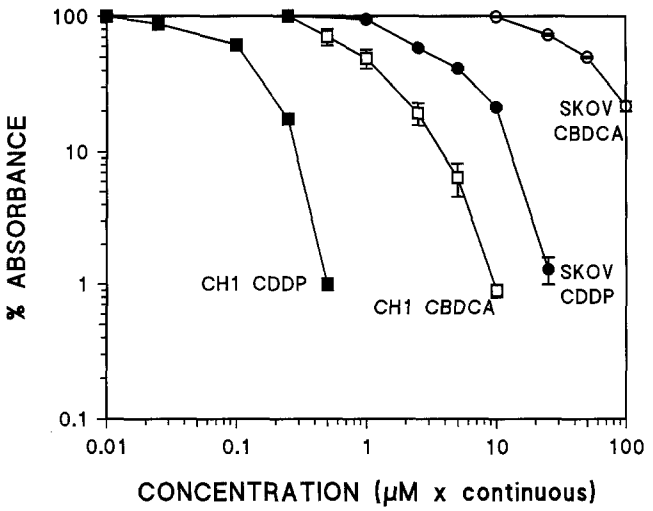

Fig. 1. In vitro sensitivity (96 h exposure, SRB assay) of the SKOV-3 (*circles*) and CH1 (*squares*) cell lines to cisplatin (*CDDP, closed symbols*) and carboplatin (*CBDCA, open symbols*)

plexes [20–23]. In addition, from the growth-delay data, we determined specific growth-delay values [37, 38] (an estimate of the number of volume-doubling times by which growth is delayed).

Results

In vitro findings

Table 2 shows the in vitro sensitivity of the eight lines in terms of IC_{50} values to 96 h exposure to the four reference platinum drugs. In molar terms, cisplatin and tetraplatin were generally more potent than carboplatin or iproplatin. As determined by tritiated thymidine incorporation or SRB staining, there appeared to be a large spread in sensitivity to each agent across the cell lines. In general terms, the HX/62 and SKOV-3 cell lines were relatively resistant to the agents, whereas the CH1 and PA1 lines were relatively sensitive. Calculation of the difference in the IC_{50} for the most sensitive vs the most resistant line produced values of 126 and 34 for cisplatin, 70 and 31 for carboplatin, 47 and 11 for iproplatin and 106 and 64 for tetraplatin as determined using the SRB and thymidine assays, respectively. This spread in sensitivity is illustrated for the CH1 and SKOV-3 cell lines using cisplatin and carboplatin in Fig. 1.

In vivo findings

Table 3 shows the corresponding responsiveness of the eight xenograft lines (note that PXN/109T/C corresponds to the CH1 cell line) in terms of growth delay, specific growth delay (SGD) and 28-day T/C analysis. Each drug was used on an equitoxic (MTD) schedule. As found in vitro, a large spread in sensitivity to cisplatin was observed across the eight lines in vivo. Again, the HX/62 and SKOV-3 lines were the most resistant, producing low growth-delay and specific-growth-delay values and correspondingly high T/C values. At the other end of the spectrum of responsiveness, the PXN/109T/C, LK1 and LK2 lines showed high growth-delay values and T/C ratios approaching zero. At an equitoxic dose that was 12.5-fold that of cisplatin, carboplatin revealed a similar pattern of response. The pattern of response to the platinum(IV) agent iproplatin was more complex, although the HX/62 and SKOV-3 tumour lines were again at the resistant end of the spectrum. The in vivo activity of tetraplatin was low as compared with the potency it displayed in vitro. Only one line, PXN/94, showed a significant growth-delay response to tetraplatin, and high T/C ratios were observed for all of the lines.

A comparative in vivo view of the difference in platinum sensitivity between the PXN/109T/C (CH1) and the SKOV-3 lines is shown in Fig. 2 (cf. the in vitro data in Fig. 1). Clearly, only a minimal reduction in tumour growth as compared with untreated tumours was observed for SKOV-3 in response to each of the platinum agents. However, in PXN/109T/C (CH1), cisplatin, carboplatin and iproplatin produced a substantial slowing of tumour growth (note the difference in time scales). Tetraplatin was essentially inactive against both of these tumour lines.

Correlation between in vitro and in vivo responses

Table 4 summarises the correlation analysis of antitumour-response data obtained for the cell lines (using IC_{50} values;

Table 2. In vitro sensitivity of the cell lines to 96 h exposure to four reference platinum drugs

Platinum drug	Assay	Sensitivity of cell lines (IC_{50}, μM)							
		HX/62	SKOV-3	OVCAR-3	PA1	CH1	PXN/94	LK2	LK1
Cisplatin	[^{3}H]-Thymidine	2.5	4.4	0.2	0.17	0.13	1.1	0.3	0.21
	SRB	12.6	4.4	0.64	0.10	0.10	3.0	0.1	0.16
Carboplatin	[^{3}H]-Thymidine	12.5	16.1	0.76	0.51	0.72	4	1.8	0.8
	SRB	70	38	14.3	0.94	1.0	31	1.1	1.5
Iproplatin	[^{3}H]-Thymidine	6.3	10.1	1.1	1.6	0.95	3.9	2.3	1.6
	SRB	70	18.5	5.1	2.4	1.5	15.2	3.1	3.1
Tetraplatin	[^{3}H]-Thymidine	1.24	10.2	1.2	0.31	0.33	0.16	0.25	0.28
	SRB	1.96	17	3.1	0.41	0.34	0.16	0.51	0.63

Data represent mean values for ≥ 3 experiments

Table 3. In vivo sensitivity of the cell lines to four reference platinum drugs

Platinum drug	Sensitivity of cell lines							
	HX/62	SKOV-3	OVCAR-3	PA1	PXN/109T/C	PXN/94	LK2	LK1
Cisplatin:								
Growth delay[a]	0.5	7.5	87.5	4.5	131	94.5	78	35
SGD[b]	0.09	1.09	6.25	2	15.6	6.2	8.2	3.6
28-day T/C	0.45	0.49	0.21	0.17	0.003	0.15	0	0.042
Carboplatin:								
Growth delay[a]	0.5	11.5	76.5	15.5	43.5	135	20.5	80
SGD[b]	0.09	1.67	5.46	7.7	5.2	8.8	2.1	8.2
28-day T/C	0.43	0.29	0.14	0.057	0.093	0.050	0.32	0
Iproplatin:								
Growth delay[a]	3	4	81.5	1	51	182	9.5	28.5
SGD[b]	0.5	0.58	5.82	0.5	6.1	11.9	1	2.9
28-day T/C	0.45	0.35	0.032	0.55	0.095	0.011	0.51	0.077
Tetraplatin:								
Growth delay[a]	1.0	1.5	4.5	0	2	73.5	1	–
SGD[b]	0.18	0.22	0.32	0	0.24	4.83	0.1	–
28-day T/C	0.40	0.58	0.56	0.68	0.54	0.54	0.95	–

[a] Time in days required for tumours in treated animals to double in volume minus that required for control tumours

[b] Specific growth delay $= \frac{T_2 - T_1}{T_1}$, where T_2 represents the time required for treated tumours to double in volume and T_1 represents that required for control tumours

Table 4. Correlation analysis of in vivo versus in vitro antitumour assessment

Drug	Growth delay[a]		28-day T/C ratio[b]	
	r	*P*	*r*	*P*
SRB assay:				
Cisplatin	–0.50	0.21	0.77	0.025*
Carboplatin	–0.20	0.64	0.67	0.07
Iproplatin	–0.14	0.74	0.29	0.48
Tetraplatin	–0.23	0.62	–0.16	0.77
[^{3}H]-Thymidine assay:				
Cisplatin	–0.54	0.17	0.88	0.004*
Carboplatin	–0.43	0.29	0.7	0.05
Iproplatin	–0.19	0.65	0.29	0.48
Tetraplatin	–0.21	0.64	–0.14	0.76

[a] Time in days required for tumours in treated animals to double in volume minus that required for control tumours

[b] 28-day T/C ratio and growth delay were calculated for cisplatin at 8 mg/kg, for carboplatin at 100 mg/kg, for iproplatin at 60 mg/kg and for tetraplatin at 8 mg/kg

* Statistically significant correlation

SRB and tritiated thymidine assays) vs the xenografts (growth delay and T/C ratio) using each of the two methods of assessment. Table 4, which compares the in vivo data obtained using the SRB assay, shows a positive, statistically significant ($P = 0.025$) correlation for cisplatin in terms of 28-day T/C ratios. Positive correlations were also apparent for carboplatin (for which the r value of 0.67 just failed to reach statistical significance) and iproplatin. However, for tetraplatin, there was no clear relationship between the in vitro and the in vivo results. This analysis is illustrated in Fig. 3 for all four agents, with each symbol representing one tumour line. The figure highlights both the general lack of activity of tetraplatin in vivo (all T/C values being greater than 0.4) and the poor correlation with the in vitro findings. A similar pattern was revealed (Table 4) by a comparison of the T/C ratios determined using the thymidine incorporation assay; again, a statistically significant positive correlation was observed for cisplatin ($r = 0.88$, $P = 0.004$) and, this time, for carboplatin as well ($r = 0.7$, $P = 0.05$). A correlation analysis of the results obtained by the two in vitro assays (SRB vs thymidine) gave a mean correlation coefficient and standard error of 0.76 ± 0.09 for the four drugs.

The correlation analysis involving growth-delay values was less clear. All four drugs produced negative correlation coefficients (i.e. the lower the in vitro IC_{50} value, the higher the growth delay), which is not surprising. As for the 28-day T/C analysis, cisplatin produced the best correlation. A comparison of the results obtained using the two xenograft assays produced a mean correlation coefficient and standard error of 0.57 ± 0.13 for the four drugs.

Data were also analysed in terms of specific-growth-delay values. A comparison of the results obtained using the SRB assay revealed correlation coefficients of –0.54 for cisplatin, –0.56 for carboplatin, –0.24 for iproplatin and –0.21 for tetraplatin. The respective values found in a comparison of the data obtained using with the thymidine uptake assay were –0.55, –0.68, –0.29 and –0.2. Therefore, although these correlation coefficients were generally a little higher than those found in comparisons of growth-delay values, the pattern of response was the same.

Discussion

A disease-oriented approach to anticancer drug discovery involving pairs of human in vitro cell lines and xenograft

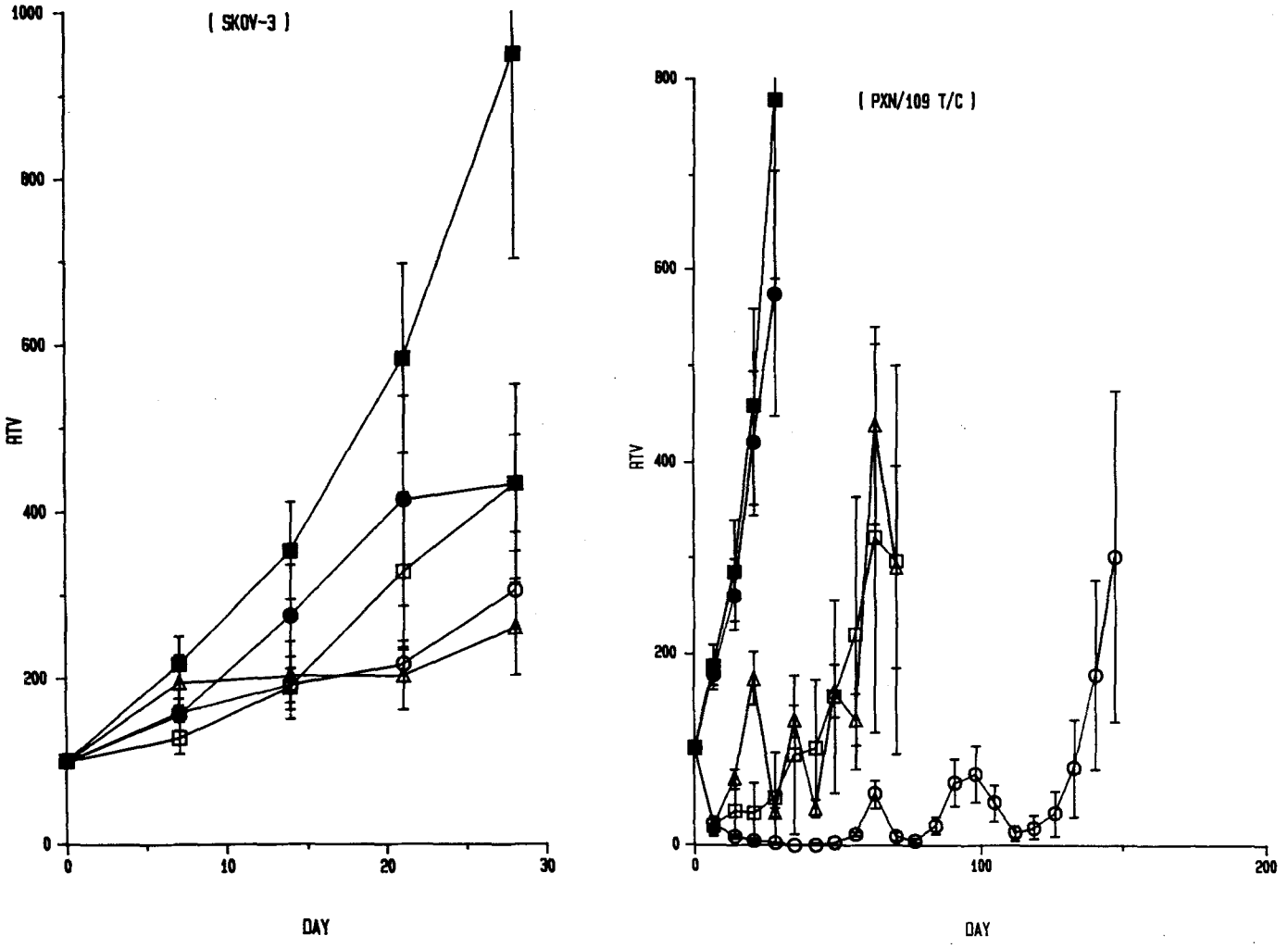

Fig. 2 a, b. Typical in vivo dose-response curves for the **a** SKOV-3 and **b** PXN/109T/C (in vivo counterpart of the CH1 cell line) xenograft lines to maximum tolerated doses and equitoxic i.p. doses of cisplatin at 8 mg/kg (*open circles*), carboplatin at 100 mg/kg (*open squares*), iproplatin at 60 mg/kg (*open triangles*) and tetraplatin at 8 mg/kg (*closed circles*). $n = 6$ for treated groups and $n = 10$ for controls (*closed squares*). *RTV*, Relative tumour volume; *error bars*, SEM

panels of a number of tumour types has recently been implemented at the National Cancer Institute [4]. At present it is too early to draw any conclusion as to whether this approach provides a reliable means of predicting for clinical antitumour activity. Our anticancer drug-development programme is aimed at broadening the clinical utility of a defined structural class of agent, namely, platinum coordination complexes. The disease-oriented approach we used in the present study focuses on ovarian carcinoma, which responds well to platinum-based chemotherapy. Using parallel cell lines and xenografts, we investigated the correlation in antitumour activity observed between these two screening systems for four established platinum drugs.

The comparative advantages and disadvantages of in vitro versus in vivo preclinical anticancer-drug evaluation are well known. Obviously, in vitro screening is much less time-consuming, easier to automate and cheaper than the use of animal tumour models, particularly nude mice. However, the xenograft model does afford information on the antitumour activity and toxicity of drugs as well as detecting those compounds requiring metabolic activation. Ovarian carcinoma xenografts have previously been used by Boven and co-workers [2, 3] to evaluate platinum complexes. The key issue relates to their respective abilities to predict reliably for clinical efficacy. In the establishment of continuous, morphologically stable cell lines and histologically stable xenograft lines that are amenable to serial passage, a significant selection process has most likely occurred in both instances from the original heterogeneous tumour tissue. However, a number of reports support the view that the level of chemotherapeutic responsiveness of tumours in humans is broadly reflected in corresponding human tumour xenografts [37, 38]. Indeed, using ovarian carcinoma xenografts, we have shown that in eight of nine lines, the therapeutic response of the xenograft to platinum-based chemotherapy reflected that of the corresponding patient's tumour [22]. In another study in which patient-xenograft comparisons were undertaken in ovarian cancer, four correlations of seven were positive [15].

The data we obtained using eight pairs of lines revealed a strong positive correlation between in vitro and in vivo antitumour responses for cisplatin and carboplatin, but the correlation for iproplatin and tetraplatin was much poorer. This correlation was apparent according to two indepen-

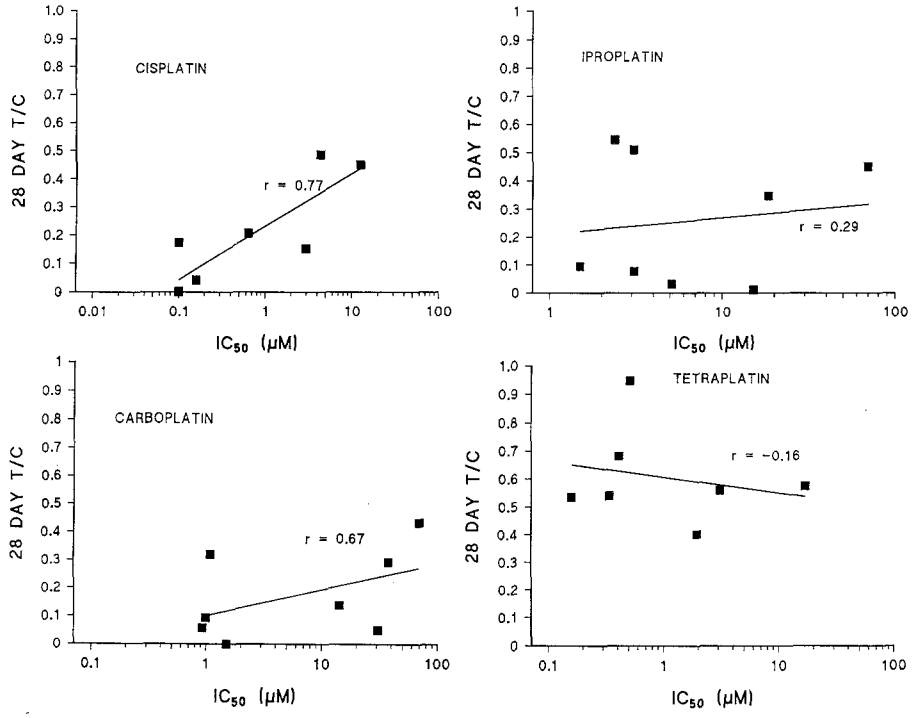

Fig. 3. Correlation between the in vitro chemosensitivity (IC_{50} value, SRB assay) and the corresponding in vivo response (28-day T/C ratio) to the four reference platinum drugs for the eight human ovarian carcinoma lines. Each symbol represents one cell line; *r* values were calculated using regression analysis

dent methods of assessment used in vitro and in vivo, although only one schedule of administration was used in vivo. Therefore, especially for cisplatin and carboplatin, tumour lines that were relatively resistant in vitro (e.g. HX/62 and SKOV-3) were most refractory to treatment in vivo, and vice versa (e.g. CH1/PXN109T/C). One possible exception was the pair of PA1 lines. Although this line was consistently among the most sensitive in vitro, it showed little response as a xenograft. The probable reason for this is the xenografted tumour's extremely short doubling time of only 2 days (see Table 1). This is reflected in the low T/C values obtained for cisplatin and carboplatin (0.17 and 0.057, respectively), which produced very little growth delay (4.5 and 15.5 days, respectively). It is conceivable that a daily administration schedule might be more appropriate for such a rapidly proliferating tumour.

It is noteworthy that the two agents producing a poorer correlation (iproplatin and tetraplatin) are platinum(IV) complexes. Previous studies using platinum(IV) complexes have shown that they are essentially inert in terms of binding to DNA (the generally accepted target for cell kill [34]). It has been shown that both iproplatin [30, 31] and tetraplatin [16] require activation via reduction to platinum(II) species before they react with DNA. The results obtained for tetraplatin in xenografts were disappointing in view of the drug's observed in vitro potency, which was similar to that seen for cisplatin. However, as noted above for the PA1 tumour, the correlation might have been better had a different schedule of in vivo tetraplatin administration been used. In addition, as reported previously by Hills et al. [24] and recently by Perez et al. [32], the pattern of in vitro response to tetraplatin across the cell lines was markedly different from that produced by the other platinum agents. In particular, among the eight cell lines used in this study, PXN/94, which was one of the most cisplatin-resistant lines, showed a strikingly differential sensitivity to tetraplatin. Our preliminary data suggest that the abnormal sensitivity of this line is due at least in part to an increased intracellular accumulation of tetraplatin. Although the in vivo activity of tetraplatin was poor, it is noteworthy that the only line to show a marked response was the PXN/94 xenograft (a growth delay of 73.5 days and an SGD value of 4.83 vs values of 94.5 days and 6.2, respectively, for cisplatin). Tetraplatin is currently undergoing phase I clinical evaluation, largely based on its activity against cisplatin (acquired)-resistant rodent tumour lines [41]. It will be particularly informative to see how these disparate predictions for the drug's activity between the human tumour xenografts described herein and cisplatin (acquired)-resistant mouse leukaemias are reflected by the clinical activity of tetraplatin.

In advanced ovarian cancer, cisplatin and carboplatin have been shown to produce similar clinical response rates in the region of 50% [27, 33]. Moreover, there is little clinical evidence to suggest non-cross-resistance between these two agents [11, 17]. Furthermore, the same pattern of shared cross-resistance has been observed for iproplatin and cisplatin [36, 40]. Our preclinical data generally show a similar pattern. Across the eight tumour lines tested, cisplatin and carboplatin produced the same pattern of response (supported by similar in vitro/in vivo correlation coefficients). Again, following treatment with iproplatin, the two cisplatin-refractory tumours (HX/62 and SKOV-3) showed little response either in vitro or in vivo. However, there was also some evidence of differential sensitivity in some xenografts that responded to these three drugs. For example, the LK2 tumour was responsive to cisplatin but markedly less so to carboplatin and iproplatin, whereas in PXN/94, carboplatin and iproplatin were somewhat more effective than cisplatin.

In summary, the correlation between the in vitro and the in vivo antitumour activity was dependent on the structure

of the platinum complex. In view of this observation, it would appear appropriate to use both cell lines and companion xenografts as disease-oriented models for the preclinical evaluation of novel platinum agents. Moreover, when these models are used together, the cell lines are valuable for the elucidation of cellular and molecular mechanisms of platinum drug sensitivity/resistance, whereas the companion xenografts may be used to study pharmacological/pharmacokinetic mechanisms of resistance. In particular, the HX/62 and SKOV-3 tumour models, which were derived from patients who had not received prior platinum therapy and generally proved to be the most platinum-resistant tumours in vitro and in vivo, represent useful evaluation models for the discovery of novel broad-spectrum platinum drugs. In addition, we are presently using platinum-sensitive tumour lines such as the CH1 (PXN109T/C) line to generate cell line and xenograft models of acquired platinum resistance.

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