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Selective potentiation of platinum drug cytotoxicity in cisplatin-sensitive and -resistant human ovarian carcinoma cell lines by amphotericin B

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Abstract Resistance to the clinically used platinum-based drugs cisplatin and carboplatin represents a major limitation to their clinical effectiveness. Using cisplatin-sensitive and -resistant human ovarian carcinoma cell lines previously characterized in terms of their major underlying mechanisms of resistance, we attempted to potentiate the cytotoxic effects of cisplatin and carboplatin using the clinically used antifungal agent amphotericin B (AmB). Using non-toxic concentrations of AmB (up to 15 μg/ml) and continuous exposure to cisplatin, a concentration-dependent selective potentiation (maximum of 3.2-fold) of cisplatin cytotoxicity was observed in two cisplatin-resistant cell lines (41McisR6, acquired resistant, and HX/62, intrinsically resistant). In both these cisplatin-resistant cell lines, previous studies have shown resistance to be due primarily to reduced platinum uptake. Notably, no significant potentiation was observed in the parent 41M cell line, in the intrinsically resistant SKOV-3 cell line (where reduced drug accumulation plays only a partial role in determining resistance) or in a pair of cell lines (CH1 and its acquired-resistant variant CH1cisR6) where reduced drug uptake does not play any role in determining resistance. The potentiating effect of AmB was lower with carboplatin and not significant in all cell lines. Platinum uptake following a 2-h exposure of cells to cisplatin was enhanced 3.5-fold in 41McisR6 cells (producing platinum levels similar to those obtained in the parental line) and 1.7-fold in 41M cells by the concomitant exposure to AmB. These data indicate that the potentiation of cisplatin (and carboplatin) cytotoxicity by AmB is not due to a generalized membrane disruption, as effects were observed only in resistant lines where reduced drug transport was apparent. Moreover, AmB did not increase the cytotoxicity of JM216 [bis-acetatoammine(cyclohexylamine)dichloroplatinum (II)], a recently developed, more lipophilic orally active platinum drug, in the 41M/41McisR6 lines. JM216 has previously been shown to circumvent acquired cisplatin resistance due to decreased drug uptake. In vivo, however, using the HX/62 xenograft, AmB (at its maximum tolerated dose of 20 mg/kg; q7d × 4 schedule) did not enhance the antitumour effect of carboplatin (at its maximum tolerated dose of 80 mg/kg; q7d × 4 schedule).

Key words Platinum · Modulation · Amphotericin B

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Introduction

Cisplatin has been widely used in cancer chemotherapy alone or in combination with other anticancer agents and has been shown to demonstrate activity against several tumours such as testicular, ovarian, head and neck, and small-cell lung carcinoma [15]. However, the significant side effects of cisplatin, which include nephrotoxicity, myelosuppression, nausea and vomiting, often limit its therapeutic efficacy [30]. The need for analogues with reduced nephrotoxicity was achieved in the form of carboplatin, which is now in clinical use worldwide [7, 18]. However, carboplatin appears to possess a spectrum of antitumour activity and a cross-resistance profile similar to those of cisplatin [4, 6].

As with many other cytotoxic drugs, the clinical effectiveness of the currently available platinum-based drugs is often limited by the development of resistance. Cellular resistance may either be present before any treatment (intrinsic) or develop after any initial response (acquired). The mechanisms of cisplatin/carboplatin resistance are often multifactorial and include reduced drug accumula-

tion, increased intracellular detoxification, reduced DNA damage and enhanced DNA repair [1, 21]. Following the identification of these various mechanisms of resistance, several studies have attempted to reverse resistance using numerous non-chemotherapeutic agents [e.g. D,L-buthionine sulfoximine (BSO) to inhibit the synthesis of glutathione (GSH), calcium channel blockers and calmodulin inhibitors; see [36] for a review).

Many studies have consistently reported that reduced cisplatin accumulation is a common mechanism of resistance in a variety of cell lines [1, 5, 14]. In our recent studies, we have shown that reduced drug uptake plays a major role in the mechanism of acquired resistance to cisplatin in one of our cisplatin acquired-resistant human ovarian cell lines, 41McisR6 [16], and in the intrinsically resistant HX/62 cell line [27]. Reduced uptake has been shown to play a partial role in another intrinsically resistant cell line, SKOV-3 [27]. However, in other acquired cisplatin-resistant cell lines (such as CH1 and CH1cisR6), reduced drug uptake does not play a role in determining resistance [12].

One agent that has been shown to potentiate the cytotoxicities of some chemotherapeutic drugs (including cisplatin) both in vitro and in vivo is the clinically important polyene antifungal drug amphotericin B (AmB) [24, 28, 29, 31]. AmB has been shown to decrease intracellular potassium ions resulting from modifications of membrane permeability. It has been assumed that the AmB-induced increase in membrane permeability is mediated through binding to sterol molecules and formation of aqueous channels [25, 32]. In the present study, we investigated the effects of AmB on the cytotoxicities of three platinum agents, cisplatin, carboplatin and the recently developed orally active platinum drug JM216 (currently in phase I clinical trial) [22], in the two cisplatin-sensitive human ovarian carcinoma cell lines 41M and CH1, described above, and their corresponding cisplatin acquired-resistant variants 41McisR6 and CH1cisR6, and in two intrinsically cisplatin-resistant lines, HX/62 and SKOV-3. Intracellular drug accumulation following 2 h exposure to cisplatin in the 41M and 41McisR6 cells was determined in combination with AmB. We also attempted to potentiate the antitumour activity of carboplatin by AmB in vivo in an intrinsically resistant xenograft, HX/62.

Materials and methods

Drugs

Cisplatin, carboplatin and JM216 were synthesized and obtained from the Johnson Matthey Technology Centre (Reading, Berkshire, UK). AmB and sulforhodamine B (SRB) were purchased from Sigma Chemicals UK Ltd.

Cell lines

Details of the establishment and biological characterization of the human ovarian carcinoma cell lines used in this study, 41M, CH1, HX/62 and SKOV-3, have been described previously [9]. The 41M cell line was derived from a previously untreated patient, CH1 was established from a patient who had prior cisplatin and carboplatin therapy, HX/62 was from a patient who had received radiotherapy and radium treatment and SKOV-3 was from a patient who had received thiotepa. Sublines of approximately 6-fold resistance to cisplatin were generated from 41M and CH1 (41McisR6 and CH1cisR6, respectively) by continuously exposing cells to increasing concentrations of drug (up to 1 μM) over a 15-month period [12].

All cells were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (Imperial Laboratories, Andover, UK), 50 μg gentamicin/ml, 2 mM L-glutamine, 10 μg insulin/ml and 0.5 μg hydrocortisone/ml in a 10% CO_2/90% air atmosphere. Cells were periodically checked and were found to be free of *Mycoplasma*.

Assessment of cytotoxicity

Cisplatin and JM216 were dissolved immediately before use in 0.9% saline at 500 μM. Carboplatin and AmB were dissolved in sterile water at 1 mM and 5 mg/ml, respectively.

The SRB assay was used to assess the cytotoxicity of the drugs. This was performed as described previously [26]. Briefly, single viable cells were seeded into 96-well microtitre plates (1×10^4 cells/well in 160 μl of growth medium). Drugs were added to quadruplicate wells after an overnight attachment period, and cells were exposed for either 2 or 96 h. After a 2-h exposure, the cells were consecutively washed with phosphate-buffered saline (PBS, pH 7.2) and medium at 37° C. Fresh medium was then added to the cells and the plates were further incubated for 96 h. In preliminary experiments, cells were exposed to various concentrations of AmB to determine non-toxic doses for the drug combination studies. In these combination experiments, cells were concomitantly exposed (2 or 96 h) to different concentrations of platinum agent and AmB. The basic amino acid content per well was then analyzed using 0.4% SRB in 1% acetic acid as described previously [26].

Intracellular platinum accumulation

Exponentially growing cells ($1-4 \times 10^6$) were treated with various concentrations of cisplatin in the absence/presence of AmB at 15 μg/ml for 2 h. Immediately after exposure, cells were washed with 3×25 ml PBS, scraped, harvested in 0.5 ml PBS and sonicated (Soniprep 150; Fisons, Loughborough, UK). All the above procedures were carried out at 4° C. The intracellular platinum content was determined using flameless atomic absorption spectrometry (Perkin Elmer models 1100B and HGA 700). The protein content was analyzed according to Lowry et al. [17]. Cellular platinum levels were expressed as nanomoles of platinum per milligram of protein.

Tumour line

The intrinsically cisplatin-resistant HX/62 cell line was grown in vivo in female nude (nu/nu) mice (age, 6–8 weeks) by subcutaneous injection of 5×10^6 cells (in 0.2 ml cell suspension) and subsequent passage from the solid tumour. Animals were housed in a Thorens maximiser and were maintained on a Labsure 21% protein diet with constant access to autoclaved tap water. The biological properties of this tumour have been described previously [11]. It has a doubling time of approximately 6 days.

Assessment of antitumour activity

Mice bearing comparably sized tumours (maximal diameter, approx. 8 mm) were randomised into four groups ($n = 6$) of controls, AmB

alone, carboplatin alone and the combination. Tumour diameters (a, longest diameter; b, longest diameter at right angles to diameter a) were measured with a slide calliper 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).

Initially, the maximum tolerated dose (MTD) of AmB was determined as 20 mg/kg (given i.p. in saline; q7d × 4 schedule). Carboplatin, dissolved immediately prior to use in water, was given i.p. at the previously determined MTD of 80 mg/kg (q7d × 4 schedule) [8]. In the combination studies, AmB was given prior to carboplatin.

Results were analyzed in terms of both growth delay (difference in the time required for control versus treated tumours to double in volume) and 28-day T/C, the ratio of the mean relative tumour volume of treated groups to that of control groups on day 28 post-treatment.

Where appropriate, statistical significance was tested using a two-tailed Student's *t*-test.

Results

In vitro studies

Initial experiments were performed to determine the maximal non-inhibitory concentration of AmB (both 2-h and 96-h exposures) against all of the cell lines studied (Fig. 1 A for 41M pair; Fig. 1 B for CH1 pair; data for HX/62 and SKOV-3 not shown). Two equimolar non-inhibitory concentrations were then selected for paired lines as follows: 41M/41McisR6, 5 and 15 μg/ml; CH1/CH1cisR6, 1 and 5 μg/ml. For HX/62 and SKOV-3, 5- and 15-μg/ml concentrations of AmB were used. The concentrations of AmB used in all cell lines in subsequent experiments conferred a maximal growth-inhibitory effect of up to approximately 15%; correction for any inhibition observed was performed by normalising to control + AmB alone values.

The effects of concomitant exposure to AmB (for either 2 or 96 h) at the non inhibitory concentrations on cisplatin cytotoxicity were evaluated in these cell lines. Following 96 h exposure to AmB at 5 or 15 μg/ml, the resistance factors for the 41McisR6 line were significantly reduced in a concentration-dependent manner from 5.1 to 2.7 ($P < 0.05$) and 1.4 ($P < 0.01$), respectively (Fig. 2 A). Interestingly, this sensitizing effect was observed only in the resistant variant 41McisR6 and not in the parent 41M line (Table 1). However, following a 2-h exposure, some potentiation of cisplatin cytotoxicity was apparent in the parental 41M line (1.8-fold), although the effects remained much greater in the 41McisR6 cells (8.1-fold) at the 15-μg/ml concentration of AmB (Table 1). AmB (at 15 μg/ml) was also capable of significantly enhancing cisplatin cytotoxicity (3.2-fold; $P < 0.05$) in the HX/62 cells following 96 h exposure; the 50% growth-inhibitory concentrations (IC_{50} values) were 11.9 ± 3.8 μ*M* for cisplatin alone, 4.5 ± 0.5 μ*M* for cisplatin plus AmB at 5 μg/ml and 3.7 ± 0.5 μ*M* for cisplatin plus AmB at 15 μg/ml. For the SKOV-3 cell line, a 2-fold (non-significant) potentiation of cisplatin cytotoxicity by AmB (at 15 μg/ml) was observed following 96 h exposure; the IC_{50} values were 2.3 ± 0.6 μ*M* for cisplatin alone, 1.8 ± 0.1 μ*M* for cisplatin plus AmB at 5 μg/ml and 1.2 ± 0.4 μ*M* for cisplatin plus AmB at 15 μg/ml. For CH1 and CH1cisR6 cells, however, AmB (at 1 or 5 μg/ml) was not capable of potentiating cisplatin cytotoxicity following 2 or 96 h exposure (Table 1). Although there was an apparent increase in resistance factors in the presence of AmB, this did not reach statistical significance (Fig. 2 B).

Cells were then treated with carboplatin in combination with AmB for 96 h in these cell lines. The results are shown in Table 2. Although the trend in potentiation of cytotoxicity by AmB was similar to that observed for cisplatin (potentiation occurring only in the 41McisR6 cell line), these effects did not reach significance. Furthermore, with the HX/62 cell line, the potentiation by AmB (at 15 μg/ml) was lower with carboplatin (and did not reach significance)

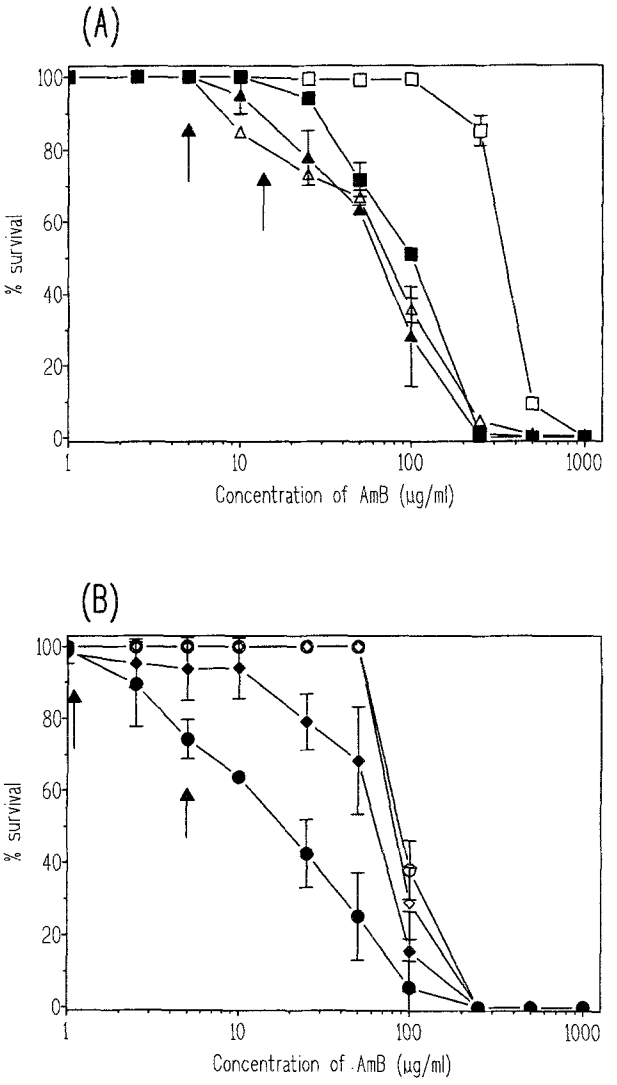
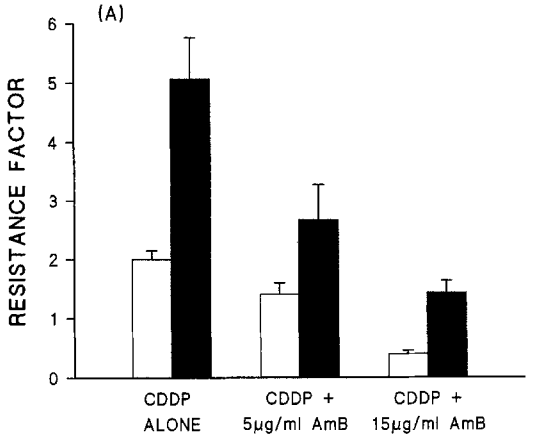


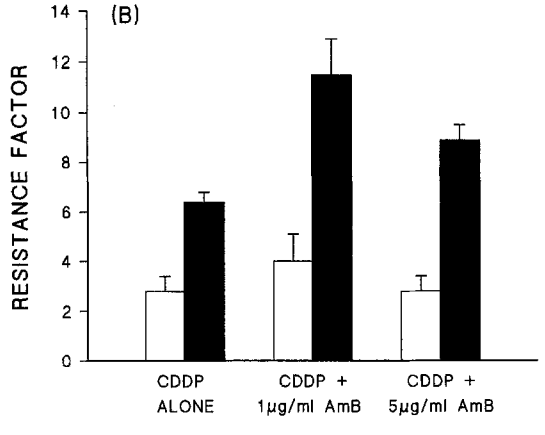
Fig. 1 A, B Assessment of cytotoxicity for AmB against the **A** 41M (*triangles*) and 41McisR6 (*squares*) cell lines and the **B** CH1 (*circles*) and CH1cisR6 (*diamonds*) cell lines following exposure for 2 (*open symbols*) and 96 h (*closed symbols*). *Arrows* indicate the non-inhibitory concentrations of AmB used in 41M/41McisR6 (5 or 15 μg/ml) and CH1/CH1cisR6 (1 or 5 μg/ml) cells. Points represent mean values; error bars indicate the SD ($n = 3$ experiments). The SD was less than the symbol size where not visible

Table 1 In vitro sensitivity profile of 41M/41McisR6 and CH1/CH1cisR6 cells following concomitant exposure for 2 and 96 h to cisplatin and AmB. Data represent mean values ± SD for 3 experiments

41M/41McisR6:				
Treatment	2-h IC_{50} (μ*M*)		96-h IC_{50} (μ*M*)	
	41M	41McisR6	41M	41McisR6
Cisplatin	3.8±0.6	7.3±1.0	0.3±0.03	1.5±0.08
Cisplatin + AmB (5 μg/ml)	3.7±0.6	5.0±0.2	0.3±0.06	0.8±0.14
Cisplatin + AmB (15 μg/ml)	2.1±0.5	0.9±0.2	0.3±0.05	0.5±0.1
CH1/CH1cisR6:				
Treatment	2-h IC_{50} (μ*M*)		96-h IC_{50} (μ*M*)	
	CH1	CH1cisR6	CH1	CH1cisR6
Cisplatin	2.6±0.9	7.5±3.9	0.08±0.01	0.62±0.1
Cisplatin + AmB (1 μg/ml)	1.9±0.6	8.1±4.2	0.07±0.02	1.07±0.4
Cisplatin + AmB (5 μg/ml)	4.2±0.9	7.4±3.4	0.07±0.02	0.62±0.1

Fig. 2A Effect of AmB (5 or 15 μg/ml) on cisplatin cytotoxicity expressed as resistance factors (R_f) of 41M versus 41McisR6 cells following 2 (*open bars*) and 96 h (*shaded bars*) exposure. R_f = IC_{50} cisR line/IC_{50} parent line. Data represent mean values ± SD for 3 experiments. **B** Effect of AmB (1 or 5 μg/ml) on cisplatin cytotoxicity expressed as resistance factors (R_f) of CH1 versus CH1cisR6 cells, following 2 (*open bars*) and 96 h (*shaded bars*) exposure. R_f = IC_{50} cisR line/IC_{50} parent line. Data represent mean values ± SD for 3 experiments





than that observed with cisplatin following 96 h drug exposure; the IC_{50} values were 120±62 μ*M* for carboplatin alone and 70.8±5 μ*M* for carboplatin plus AmB at 15 μg/ml. In contrast to the data obtained for cisplatin or carboplatin in the 41McisR6 cell line, there was no significant enhancement of JM216 cytotoxicity against 41M/41McisR6 at either the 5- or the 15-μg/ml concentration of AmB (Table 3).

Intracellular platinum accumulation in the 41M and 41McisR6 cells was determined following a 2-h exposure to various concentrations of cisplatin with or without AmB at 15 μg/ml. Figure 3 shows that across the range of cisplatin concentrations, the platinum levels in the presence of AmB at 15 μg/ml were an average of 1.7±0.2 and 3.5±1.1 times higher in the 41M and 41McisR6 cells, respectively, as compared with their control levels. Consistent with the cytotoxicity data, platinum accumulation in the acquired-resistant line in the presence of AmB recovered to that observed in the parental line in the absence of AmB.

In vivo studies

Both cisplatin and AmB have been shown to induce nephrotoxicity [30, 32]. Therefore, in spite of the in vitro potentiation effects being weaker with carboplatin than with cisplatin, in vivo experiments to examine the modulating effects of AmB were performed with the less nephrotoxic analogue carboplatin rather than cisplatin [7]. Indeed, preliminary experiments attempting to combine AmB with cisplatin (at 2 mg/kg; around 40% of the MTD) showed histological evidence for increased nephrotoxicity. No dose reduction was required with carboplatin. The intrinsically cisplatin-resistant HX/62 tumour line was selected, as in vitro data had shown that this was one of only two cell lines (41McisR6 being the other) where carboplatin cytotoxicity was potentiated by AmB (effects were of a similar magnitude in HX/62 and 41McisR6). The results (Fig. 4) showed that neither AmB alone, carboplatin alone nor the combina-

Table 2 Comparison of in vitro sensitivity profile between 41M/41McisR6 and CH1/CH1cisR6 cells following concomitant exposure for 96 h to carboplatin and AmB. Data represent mean values ± SD for 3 experiments

Treatment	96-h IC_{50} (μM)					
	41M	41McisR6	+Rf	CH1	CH1cisR6	R_f[a]
Carboplatin	3.0±0.7	5.4±1.4	1.8±0.1	1.3±0.3	4.4±0.6	3.5±0.4
Carboplatin + AmB (5 μg/ml)-41M pair/ AMB (1 μg/ml)-CH1 pair	3.0±0.8	4.0±0.6	1.4±0.2	1.1±0.2	4.0±0.3	3.7±0.6
Carboplatin + AMB (15 μg/ml)-41M pair/ AmB (5 μg/ml)-CH1 pair	3.2±0.6	3.3±0.8	1.0±0.1	1.1±0.3	4.0±0.6	3.7±1.9

[a] Resistance factor (IC_{50} cisR/IC_{50} parent line)

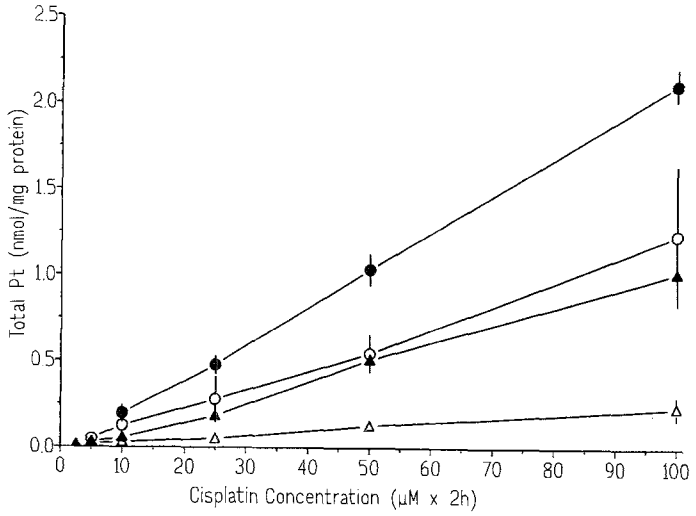
Table 3 The effect of AmB on JM216 cytotoxicity in 41M and 41McisR6 cells following 96 h exposure. Data represent mean values ± SD for 4 experiments

Treatment	96-h IC_{50} (μM)		
	41M	41McisR6	R_f[a]
JM216	0.7±0.1	0.5±0.1	0.8±0.2
JM216 + AmB (5 μg/ml)	0.7±0.1	0.5±0.03	0.7±0.2
JM216 + AmB (15 μg/ml)	0.7±0.04	0.5±0.1	0.8±0.1

[a] Resistance factor (IC_{50} cisR line/IC_{50} parent line)

tion exerted any significant antitumour effect against this refractory tumour line. The 28-day T/C values were as follows: AmB alone, 1.3; carboplatin alone (80 mg/kg; q7d × 4), 0.88; and carboplatin (80 mg/kg; q7d × 4) plus AmB (20 mg/kg; q7d × 4), 0.8. Growth delay values were –0.2, –1.8 and 0.1 days, respectively.

Fig. 3 Intracellular platinum accumulation in 41M (*circles*) and 41McisR6 (*triangles*) cell lines immediately after a 2-h exposure to various concentrations of cisplatin in the absence (*open symbols*) or presence (*closed symbols*) of AmB at 15 μg/ml. Points represent mean values; error bars indicate the SD of triplicate determinations in 2 experiments. The SD was less than the symbol size where not visible



Discussion

Many studies have reported a multifocal basis of cisplatin resistance, often involving more than one mechanism within an individual tumour cell line, and these mechanisms vary from one cell line to another. Thus far, approaches to reverse resistance have met with limited success. In this study, we selected two cell lines with acquired cisplatin resistance and two intrinsically cisplatin-resistant cell lines. Our previous results have suggested that these cell lines exhibit differing biochemical mechanisms of cisplatin resistance. In the 41McisR6 line, reduced platinum accumulation, primarily through the plasma membrane, is likely to be the major determinant of acquired resistance, whereas in the CH1cisR6 cells, resistance appears to involve effects at the DNA level [12, 16]. Reduced drug accumulation also plays a significant role in the intrinsic resistance to cisplatin in the HX/62 line and a partial role in the SKOV-3 cell line [27]. Several studies have implicated a platinum accumulation defect as an important mechanism of cisplatin resistance in a variety of cell lines [10, 19, 35]. Thus, agents that are capable of increasing intracellular platinum accumulation may be useful in attempts to overcome resistance to cisplatin. Verapamil has been reported to increase the uptake of several anticancer drugs, such as Adriamycin and vinblastine, in multidrug-resistant cells by inhibiting P-glycoprotein. However, we have shown that none of our platinum-resistant cell lines exhibits elevated levels of the P-170 glycoprotein and, moreover, that modulators of P-glycoprotein fail to enhance cisplatin cytotoxicity (Sharp et al., unpublished data).

In recent years, it has become apparent that AmB can sensitize cells to cisplatin and other platinum-containing agents [13, 20, 28, 29]. As AmB can produce pores of about 8A in diameter in the membrane, which are permeable for water, non-electrolytes and ions [3], it has been assumed that sensitization to cisplatin by AmB results from increasing intracellular platinum accumulation [13, 29]. In this study, we showed that after 96 h exposure, AmB (at 5 or 15 μg/ml) was capable, in a concentration-dependent manner, of significantly enhancing ($P < 0.05$) cisplatin cytotoxicity in the acquired-resistant variant 41McisR6 (3.2-fold) but not in the parental 41M line. Interestingly,

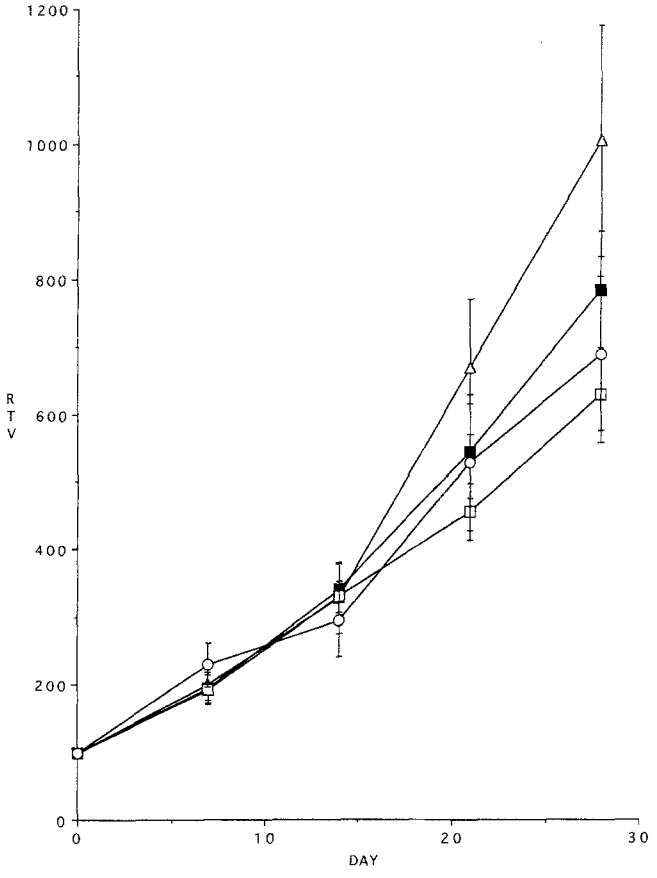


Fig. 4 Tumour growth curves generated for the human ovarian carcinoma xenograft HX/62, expressed as relative tumour volumes (*RTV*) for controls (*solid squares*), AmB alone (at 20 mg/kg; *open triangles*), carboplatin alone (at 80 mg/kg; *open circles*), and carboplatin (at 80 mg/kg) plus AmB (20 mg/kg; *open squares*). Carboplatin and AmB were given on an i.p. q7d × 4 schedule

cisplatin cytotoxicity was augmented in both the 41M (1.8-fold) and 41McisR6 (8.1-fold) cells following 2 h exposure. This correlated with the increased cisplatin accumulation observed for both cell lines after 2 h treatment (1.7-fold in 41M and 3.5-fold in 41McisR6). Hence, the AmB-induced potentiation of cisplatin cytotoxicity in the parent line appears to be mainly due to an increased drug accumulation, whereas for the 41McisR6 cells, additional mechanisms may be involved. The enhancement of cisplatin cytotoxicity by AmB was also shown in the intrinsically cisplatin-resistant HX/62 cell line (3.2-fold; $P < 0.05$) and in the SKOV-3 cell line (2-fold), although statistical significance was not reached for SKOV-3. For the CH1 and CH1cisR6 cells, where the mechanism of acquired resistance to cisplatin appears to occur at the DNA level, AmB (1 or 5 μg/ml) was not capable of potentiating cisplatin cytotoxicity following 2 and 96 h exposure. These results suggest that the effect of AmB at the cellular level is much more selective and complex than the simple production of pores in the membrane of the cell. This suggestion is consistent with other recent observations of cell line specificity for AmB potentiation of cisplatin cytotoxicity [13, 29].

The potentiation effect of AmB appeared lower with carboplatin than with cisplatin, although, as with cisplatin, the 41McisR6 and HX/62 cell lines were the only lines where potentiation was observed (SKOV-3 was not included in the carboplatin studies), but these effects did not reach statistical significance. JM216, which possesses lipophilic axial acetato ligands, is capable of circumventing cisplatin resistance in 41McisR6 cells by increasing the drug uptake [16]. Significantly, AmB was not capable of potentiating the cytotoxicity of JM216 in either the parent 41M or the resistant variant 41McisR6. These results suggest that AmB appears to potentiate platinum drug sensitivity selectively in resistant cells exhibiting reduced drug accumulation defects. Interestingly, Morikage et al. [29] have recently shown a similar lack of sensitization by AmB with another lipophilic platinum analogue, Ormaplatin [tetraplatin, (1,2-diaminocyclohexanetetrachloroplatinum (IV)].

The in vitro effects of AmB observed in this study occurred mainly at a concentration of 15 μg/ml. Clinically, AmB levels of around only 4 μg/ml have been obtained [2]. Moreover, AmB has been shown to induce nephrotoxicity in man [32]. Indeed, our murine data showed histological evidence for an increase in cisplatin-induced nephrotoxicity in the presence of AmB. Therefore, in spite of less in vitro potentiation being observed, the less nephrotoxic platinum drug, carboplatin, was selected for use in combination with AmB. In light of these findings, we evaluated the antitumour effects of carboplatin in combination with AmB using the cisplatin-resistant HX/62 tumour in vivo. Previous studies have shown that the in vivo responsiveness of this tumour correlates with the in vitro findings in that it is highly refractory to various platinum-based drugs, including cisplatin and carboplatin [8, 11]. Other studies have shown that various degrees of potentiation of several anticancer drugs (e.g. Adriamycin, melphalan) can be achieved with AmB in mice bearing AKR leukaemia [23, 24]. However, the results we obtained with the combination were disappointing in that AmB (at the MTD) did not significantly enhance the antitumour activity of carboplatin alone. Although plasma levels of AmB were not measured in this study, it appears likely that the lack of sensitization in vivo was due to both less potentiation being achievable and/or inadequate tumour levels of AmB being reached (possibly because of extensive protein binding). In addition, other mechanisms of resistance to cisplatin/carboplatin may be operating [e.g. enhanced DNA repair or increased levels of glutathione (GSH)]. Previously, we have observed relatively high GSH levels in the HX/62 and SKOV-3 cell lines [26].

In conclusion, although additional in vitro experiments with AmB might shed further light on the important question of how cisplatin and other platinum-based drugs enter cells (e.g. passive diffusion versus facilitated transport), the in vivo usefulness of AmB/platinum combination therapy might be limited due to inadequate plasma levels of AmB being achievable plus overlapping nephrotoxic effects of the two drugs. However, recent clinical data obtained using sonicated liposomal preparations of AmB, where peak plasma levels of 10–20 μg/ml were achieved, may

provide a strategy whereby certain tumours could be sensitized to carboplatin [33, 34].

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