A Novel trans-Platinum Coordination Complex Possessing in Vitro and in Vivo Antitumor Activity

Lloyd R. Kelland, Christopher F. J. Barnard, Kirste J. Mellish, Mervyn Jones, Phyllis M. Goddard, Melanie Valenti, Alexander Bryant, Barry A. Murrer, and Kenneth R. Harrap


ABSTRACT

As part of a drug discovery program to discover more effective platinum-based anticancer drugs, a series of platinum complexes of trans coordination geometry centered on trans-ammine(cyclohexylaminedichlorodihydroxy)platinum(IV) (JM335) has been evaluated in vitro against a panel of cisplatin-sensitive and cisplatin-resistant human tumor cell lines (predominantly ovarian). In vitro, against 5 human ovarian carcinoma cell lines, JM335 was comparably cytotoxic to cisplatin itself and over 50-fold more potent than transplatin (mean 50% inhibitory concentrations: JM335, 3.1 μM; cisplatin, 4.1 μM; transplatin, 162 μM). With the use of seven pairs of human tumor cell lines (parent and subline with acquired resistance to cisplatin and encompassing all of the known major mechanisms of resistance to cisplatin) JM335 exhibited a different cross-resistance pattern to that of its cis isomer (JM149). JM335 showed non-cross-resistance in six of the seven resistant lines, cross-resistance in the A2780cisR line possibly being associated with high levels of glutathione. Preliminary intracellular DNA binding studies showed that in contrast to transplatin, JM335 was efficient at forming DNA-DNA interstrand cross-links. In vivo, JM335 produced growth delays in excess of 15 days against 4 of 6 human ovarian carcinoma xenografts and was unique among the complexes studied in retaining some efficacy against a cisplatin-resistant subline of the murine ADJ/PC6 plasmacytoma. JM335 is the first trans-platinum complex to demonstrate marked antitumor efficacy against both murine and human s.c. tumor models and represents a significant structural lead to complexes capable of circumventing cross-resistance to cisplatin.

INTRODUCTION

Following the successful introduction into the clinic of cisplatin and the less toxic analogue, carboplatin, drug resistance represents the major limitation of these important anticancer drugs. Laboratory-based studies have revealed that a combination of reduced platinum accumulation, increased cytoplasmic detoxification (through increased levels of glutathione and/or metallothioneins), and enhanced DNA-platinum adduct removal are the major mechanisms underlying resistance to cisplatin/carboplatin (reviewed in Ref. 1). Platinum complexes based on the 1,2-diaminocyclohexane carrier ligand, such as Ormaplatin and Oxaliplatin, entered clinical trial based on their circumvention of acquired cisplatin resistance (predominantly with the use of the murine L1210 leukemia model; Ref. 2). To date, however, 1,2-diaminocyclohexane-platinum complexes have not shown marked clinical activity and, moreover, appear to suffer from the major drawback of severe neurotoxicity (3–4). We have previously described a novel class of platinum complex, the ammine/amine platinum(IV) dicarboxylates, which appear capable of largely circumventing acquired cisplatin resistance mediated at the level of the plasma membrane (5, 6). One example, the p.o. administered JM216,3 is currently undergoing Phase II clinical trials (7). A further approach to the circumvention of resistance to cisplatin might be to design novel platinum complexes which should bind to DNA in a manner distinct from that of cisplatin and carboplatin (which both possess symmetrical cis-ammine carrier ligands).

The paradigm for structure-activity relationships of the platinum-based coordination complexes is that transplatin, the trans isomer of cisplatin, is inactive as an antitumor agent (8). Nonetheless, in recent years, we and others (9–12) have pursued the rational drug design idea of activating the trans geometry of platinum complexes since their binding geometry to DNA has been shown to differ from that of cisplatin (13). This study reports the in vitro and in vivo antitumor activity against murine ADJ/PC6 and human tumor (predominantly ovarian) models both sensitive and resistant to cisplatin, and preliminary mechanistic studies on the DNA-binding properties of a novel series of trans platinum complexes centered around JM335. A comparison has been made with cisplatin and transplatin and the cis isomer of JM335 (JM149).

MATERIALS AND METHODS

In Vitro Studies

Cell Lines. Seven “parent” human ovarian carcinoma cell lines have been used (SKOV-3, OVCA-3, A2780, HX/62, PXN94, CH1, and 41M), as described previously (7). In addition, seven pairs of human tumor cell lines (parent line and derived subline with acquired resistance to cisplatin, or tetraplatin in the case of PXN94) have been used [41M/41McisR, CH1/CH1cisR, A2780/A2780cisR, OVCA-3/OVCA-3cisR, PXN94/PXN94tetR (all ovarian), GCT27/GCT27cisR (testicular nonseminomatous germ cell), and HX155/HX155cisR (cervical carcinoma)], as described previously (7), except for PXN49tetR. The parent 41M, A2780, GCT27, and HX/155 lines were all derived from previously untreated patients.

All lines grew as monolayers in Dulbecco’s modified Eagle’s medium containing 10% fetal calf serum (Imperial laboratories, Andover, UK), 50 μg/ml of gentamicin, 2.5 μg/ml of amphotericin B, 2 mM t-glutamine, 10 μg/ml of insulin, and 0.5 μg/ml of hydrocortisone in 10% CO2/90% air. Cells were periodically checked and found to be free of Mycoplasma; parent lines were used from passages 25–60. Platinum Drugs. Cisplatin, transplatin, JM149, JM334, and JM335 were synthesized by and obtained from the Johnson Matthey Technology Centre (Reading, Berkshire, UK). The structures of these agents are shown in Fig. 1.

Assessment of Cytotoxicity. Platinum drugs were dissolved immediately before use in 0.9% saline at 1 mM or 500 μM (for JM334 and JM335, respectively). Cytotoxicity was then assessed using the SRB assay as described previously (5–7). Briefly, single viable cells were seeded into 96-well microtiter plates (at concentrations between 5 × 103 and 1 × 104) well in 200 μl growth medium) and allowed to attach overnight. Agents were then added to quadruplicate wells. Agent exposure was for 96 h unless stated otherwise.

3 The abbreviations used are: JM216, bis-acetato-ammine(dichlorodicyclohexylamine)platinum(IV); JM335, trans-ammine(cyclohexylaminedichlorodihydroxy)platinum(IV); JM334, trans-ammine(dichlorocyclohexylamine)platinum(II); IC50, 50% inhibitory concentration; SRB, sulforhodamine B; GSH, glutathione; t-BSO, t-buthionine sulfoximine; ISC, interstrand cross-link.

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2 To whom requests for reprints should be addressed.
of the maximum tolerated dose to the dose required to reduce tumor mass by 90%.

**Human Ovarian Carcinoma Xenografts.** Xenografts were performed as described previously (17—18). Mice bearing comparably sized tumors (typically 8 mm, largest diameter) were treated with JM335 (administered i.p. as a sonicated suspension in arachis oil) at previously determined maximum tolerated doses (approximately 10% lethal dose) on days 0, 7, 14, and 21. Tumors were measured weekly until they had doubled their starting volume; response was assessed as described previously (17—18). Growth delays (the difference in time required for control and treated tumors to double in volume) were then determined.

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

**RESULTS**

**In Vitro Cytotoxicity.** Cytotoxicity determinations for cisplatin, transplatin, JM149, and JM335 against a panel of 5 human ovarian carcinoma cell lines are shown (Fig. 2). Across the 5 lines, transplatin was approximately 40-fold less cytotoxic than cisplatin (mean IC50 values, 162 and 4.1 μM, respectively). However, in contrast to transplatin, JM335 was comparably cytotoxic to cisplatin (mean IC50, 3.1 μM) and about 3-fold more cytotoxic than its corresponding cis isomer, JM149 (mean IC50, 10.6 μM). A broadly similar pattern of response was also observed following a short (2-h) exposure to drugs; for the SKOV-3 cell line, 2 h IC50 values were: cisplatin, 31 ± 2.7 (SD) μM; transplatin, 480 ± 61 μM; JM149, 336 ± 90 μM; and JM335, 75 ± 27 μM.

Also of interest is the observation that the range in IC50 values (i.e., most resistant to most sensitive cell line) was much greater for the two cis compounds, cisplatin and JM149 (126 and 53, respectively), than for transplatin and JM335 (7.7 and 5.4, respectively).

The ability of JM335 to circumvent cisplatin-acquired resistance in a variety of resistant human tumor cell lines is shown in Fig. 3. In addition, comparative cross-resistance data for JM149 are shown. JM335 exhibited a different cross-resistance profile than that observed for JM149; less cross-resistance was observed with JM335 against the CH1cisR, GCT27cisR, and HX/155cisR lines, and the PXN94-resistant line (which was selected for resistance using tetraplatin but also shows 6.5-fold resistance to cisplatin). In one cell line pair, A2780/
A2780cisR, greater cross-resistance to JM335 compared to JM149 was observed. Taking a resistance factor of <2 to denote non-cross-resistance, JM335 exhibited non-cross-resistance in 6 of the 7 pairs, whereas JM149 circumvented resistance in 3 (41M, OVCAR-3, and HX/155).

Previous studies have shown that the A2780cisR cell line is unique among the acquired resistant lines evaluated in this study in possessing significantly elevated glutathione levels compared to its parental line (GSH levels: nmol/mg protein, A2780 = 7.8 ± 0.7; A2780cisR = 42 ± 4.5; P < 0.01) (5, 15, 19). We have investigated the possible effect of GSH on mediating the cytotoxicity of the cis- and trans-platinum complexes studied herein by using L-BSO to reduce levels of GSH prior to exposure of cells to the complexes. As shown that increasing doses (up to the maximum tolerated dose of 4 mg/kg) induced increasing tumor growth delays. JM335 produced marked activity (growth delay, >15 days) against 4 of the 6 xenografts studied (PXN/109T/C, PXN/100, OVCAR-3, and HX/110). In addition, some activity was observed against the intrinsically cisplatin-resistant SKOV-3 (16) and the acquired cisplatin-resistant PXN/109/T/CC (18).

**DISCUSSION**

A novel trans-platinum complex, JM335, has exhibited greater in vitro cytotoxicity against human ovarian carcinoma cell lines than both transplatin (approximately 50 times as potent) and its corresponding cis congener (JM149). Compared to cisplatin and JM149,
JM335 also showed a much narrower range in cytotoxicity across the cell line panel. The chemistry (synthesis, stability, and physical properties) of JM335 and additional trans-platinum complexes will be reported elsewhere.\(^5\)

Furthermore, JM335 showed a greater ability than JM149 to circumvent acquired cisplatin resistance in six of seven resistant human tumor cell lines. Non-cross-resistance between JM335 and cisplatin has been demonstrated in acquired cisplatin-resistant cell lines where reduced platinum accumulation \([\text{cisR}]\) or resistance has been shown to be mediated mainly through either differences in reactivity with the high levels of GSH observed in this cell line. Notably, in many of the remaining acquired resistant lines, GSH levels have been shown to be similar to those present in parental lines (5, 6, 15, 19). However, because two independent groups have also shown that DNA-sequence specific ISC repair is probably involved in resistance in A2780cisR (20, 21), this could also be contributing to the cross-resistance to JM335.

We have previously described a novel platinum complex, JM216, which is currently undergoing Phase II clinical trials by p.o. administration and which has also shown promise in circumventing acquired cisplatin resistance \textit{in vitro} (7). As with JM335, JM216 was also effective in overcoming resistance in the lines where reduced accumulation represents the main mechanism of resistance \([\text{cisR}]\). Notably, however, JM335 was more effective than JM216 in cell lines where enhanced removal of or increased tolerance to platinum-DNA adducts mainly contributes to the resistance \([\text{cisR}]\). Resistance factor values were 1.9 \textit{versus} 4 for CH1/CH1cisR and 1.8 \textit{versus} 2.9 for GCT27/GCT27cisR for JM335 and JM216, respectively. With the A2780/A2780cisR pair of lines, JM216 was the more effective; resistance factor values were 7.1 for JM335 \textit{versus} 4.5 for JM216.

Recently, two other classes of novel trans-platinum complex have been described, those of structural formula \textit{trans}-\([\text{PtCl}_2(L)(L')]\), where \(L = L'\) are planar ligands such as pyridine or thiazole (9–11), and bis-substituted imino ethers of structural formula \textit{trans}-\([\text{PtCl}_2(\text{imino ether})_2]\). In common with our findings, a lower range in cytotoxicity was observed for \textit{trans}-\([\text{PtCl}_2(\text{pyridine})_2]\) compared to cisplatin (11). In addition, \textit{trans}-\([\text{PtCl}_2(\text{pyridine})_2]\) was also shown to react more slowly with GSH than transplatin itself \(\left(\text{half-life for the reaction of transplatin with a 5-fold excess of GSH was calculated as only 0.5 min}\right)\) (11). Our cytotoxicity results obtained in the presence or absence of preexposure to L-BSO \((\text{in a cell line possessing high intrinsic GSH levels})\) also suggest that although JM335 may be more susceptible to reactivity with intracellular thiol-containing species than either JM149 or cisplatin, it may be considerably less susceptible than transplatin \((\text{where, in agreement with previous findings (22), a marked potentiation in cytotoxicity was observed by depleting cellular GSH})\).

In contrast to the \textit{trans}-\([\text{PtCl}_2(\text{pyridine})_2]\) complex which has not, to date, shown activity \textit{in vivo} (9), a \textit{trans}-iminio ether platinum(II) complex showed significant \textit{in vivo} antitumor activity in mice bearing P388 leukemia \((\text{particularly when administered for a 7-day schedule})\) and retained some activity against a P388/cisplatin-resistant subline (12). However, these effects were obtained using i.p. drug administration within 1 day of i.p. implantation of tumor cells; studies using established s.c. tumors and drug administration distant from the tumor site have not yet been reported. Our studies demonstrate that JM335 possesses marked antitumor efficacy against a variety of s.c. tumor models. JM335 was active against the murine ADJ/PC6 plasmacytoma, retained some efficacy against a subline selected for resistance to cisplatin (where resistance is probably due to enhanced DNA repair and/or increased tolerance to platinum-DNA adducts \([23]\)), and showed good activity \((>15 \text{ days growth delay})\) against 4 of 6 human ovarian carcinoma xenografts. The maximum tolerated single dose of JM335 in mice bearing the ADJ/PC6 plasmacytoma was similar to that observed for cisplatin; studies investigating the pharmacological and toxicological properties of JM335 are in progress.

The reasons for the observed antitumor activity of JM335 are presently unknown, although by analogy with classical alkylating agents...
agents, at least part of the cytotoxicity observed with JM335 could be attributable to the formation of DNA-DNA ISCs. Although both cisplatin and transplatin are capable of forming DNA intrastrand and interstrand cross-links, controversy remains as to the explanation underlying the contrasting antitumor effects of the two congeners (reviewed in Ref. 13). Transplatin is stereoelectronically incapable of forming the 1,2 intrastrand d(GpG) or d(ApG) cross-links (the major adducts formed by cisplatin) (13, 24), suggesting that the differences in antitumor activity may result from the different nature of distortions induced in DNA by the various intrastrand crosslinks. Others have suggested that the inactivity of transplatin results from a high proportion of monofunctional adducts on DNA [which may rapidly react with glutathione before they can be converted to more toxic bifunctional adducts (24–25)] and/or through preferential recognition and repair of transplatin-induced DNA adducts (26). Recent studies, however, have highlighted the possible importance of ISCs and their repair in determining the cytotoxicity of cisplatin (20–21); while cisplatin preferentially forms ISCs between guanine residues (27), transplatin has recently been shown to preferentially form ISCs between guanine and complementary cytosine residues (25).

Detailed studies of the intracellular transport properties and the interaction of these platinum complexes with DNA are in progress and should shed light on the mechanism(s) underlying the above findings. Nonetheless, the preclinical antitumor properties of JM335 suggest that this agent, the first trans-platinum complex to exhibit unequivocal in vivo antitumor activity against both murine and human s.c. tumor models, represents a significant structural lead to the development of platinum complexes exhibiting non-cross-resistance to cisplatin.

REFERENCES

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