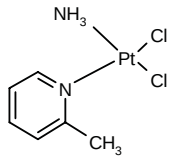
Drugs of the Future 1998, 23(10): 1062-1065
Copyright © 1998 PROUS SCIENCE
CCC: 0377-8282/98

AMD473

Antineoplastic

JM-473
ZD-0473

cis-Amminedichloro(2-methylpyridine)platinum(II)



$C_6H_7N.Cl_2Pt.H_3N$ Mol wt: 376.145

CAS: 181630-15-9

EN: 234240

Introduction

The platinum coordination complex, cisplatin [*cis*-diamminedichloroplatinum (II)], represents one of the mainstays of the drug treatment of cancer. Since its introduction into clinical practice in the mid-1970s, there has been considerable effort to discover superior platinum-containing analogs. Two broad themes of analog development have transpired: (i) improving patient quality of life through reduced side effects and oral therapy, and (ii) widening the spectrum of antitumor activity to tumors that are initially, or have become, unresponsive to cisplatin. Despite considerable effort and the description of hundreds of analogs, only around 25 compounds have entered phase I and only one, carboplatin (Paraplatin®), Bristol Myers Squibb), is widely registered for use. Two additional compounds are registered in individual countries: oxaliplatin (Eloxatin®) in France and 254-S (nedaplatin, Aqupla®) in Japan (1).

A collaborative program between Johnson Matthey, Bristol Myers Squibb and the CRC Centre for Cancer Therapeutics was established to discover and develop a platinum drug capable of oral administration, possessing less severe toxicity than cisplatin and being at least as active as cisplatin/carboplatin. This led to the development of JM216 [bis(acetato)amminedichloro(cyclohexylamine)platinum(IV); BMS 182751, Bristol Myers Squibb] which entered clinical trials in 1992 (2, 3).

The Johnson Matthey/CRC Centre for Cancer Therapeutics collaboration was continued during the early 1990s with the specific aim of discovering platinum complexes possessing activity against cisplatin-refractory disease. A mechanism-directed evaluation cascade was established whereby novel molecules were evaluated against a panel of 8 *in vitro* human ovarian carcinoma cell lines representative of intrinsic and acquired cisplatin resistance. Three pairs of cell lines were used, where in each, the major underlying mechanism of resistance had been determined. Agents were sought which circumvented resistance in all pairs of lines. Ultimately, this led to the discovery of ZD0473 (AMD473; JM473; *cis*-[amminedichloro(2-methylpyridine)]platinum(II)).

The chemical rationale for the synthesis of AMD473 was based on having a platinum compound with reduced susceptibility to inactivation by elevated intracellular thiol concentrations. A number of studies, including our own using the ovarian cell line panel, have shown that thiols, especially glutathione (GSH), represent a significant cause of resistance to cisplatin (4). In an attempt to decrease inactivation by thiols, increased steric bulk was introduced at the platinum center, thereby shifting the substitution reaction pathway more towards a dissociative rather than associative mechanism.

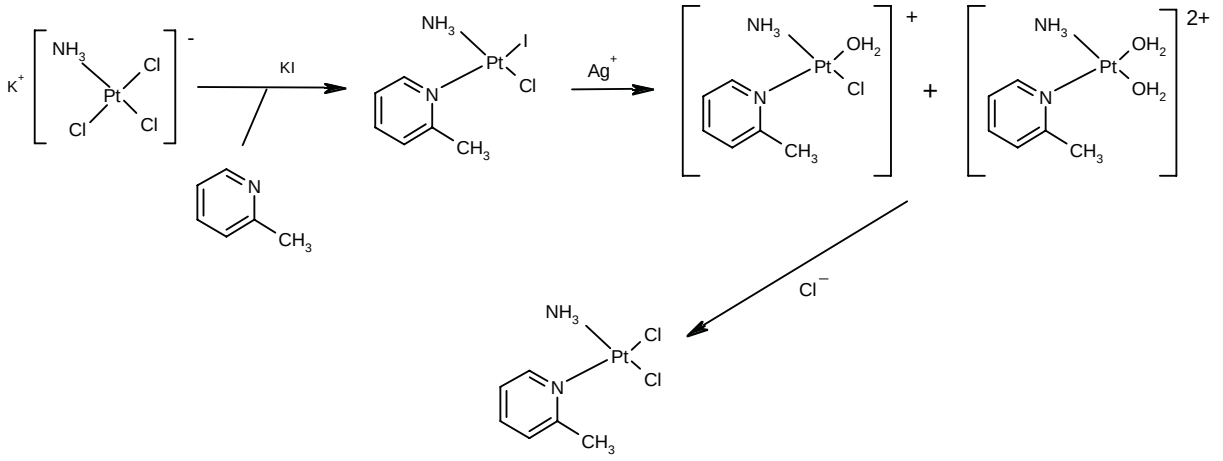
Chemistry

AMD473 is prepared by a modified Dhara synthesis (5) (Scheme 1). The procedure is similar to that described for the preparation of the platinum(II) precursor of the other mixed amine platinum drug currently in development, JM216 (6).

In the first step, potassium amminetrichloroplatinate (II) is reacted with 2-picoline in the presence of iodide at

Lloyd R. Kelland[1]* , Christopher D.J. Barnard[2]. [1]CRC Centre for Cancer Therapeutics, The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey SM2 5NG, UK; [2]Johnson Matthey Technology Centre, Blount's Court, Sonning Common, Reading RG4 9NH, UK. *Correspondence.

Scheme 1: Synthesis of AMD473



ambient temperature. The direction of substitution in platinum(II) complexes is controlled by the *trans* effect of the different ligands. Since in $[PtCl_3(NH_3)]$ the *trans* effect for Cl > NH_3, one of the *trans* pair of chloride ligands is initially substituted by iodide. Iodide has a much greater trans effect than chloride or ammine ligands and directs further substitution trans to itself. Binding of 2-picoline results in precipitation of the desired isomer $[PtCl(I)(NH_3)(2\text{-picoline})]$. In the second step, the iodide ligand is removed by reaction with silver ions in water at ambient temperature and treatment of the resulting aqua platinum complexes with chloride yields $[PtCl_2(NH_3)(2\text{-picoline})]$. This occurs with retention of stereochemistry at platinum, so yielding the pure *cis* isomer. This complex may be recrystallized from dilute hydrochloric acid.

Pharmacological Actions

In common with other platinum-containing agents, AMD473 is presumed to confer its cell killing properties by interaction with DNA to form various monofunctional and bifunctional intrastrand and interstrand cross-links. As predicted by the above chemical substitution and steric hindrance considerations, AMD473 was shown to be less reactive than cisplatin towards the sulphur-containing molecules methionine and thiourea (7). Moreover, AMD473 binding to salmon sperm DNA was significantly less affected than cisplatin when 5 mM GSH was added (8). When GSH levels were artificially raised in an ovarian cancer cell line, the degree of protection of cytotoxicity was less for AMD473 compared to cisplatin (8). Although the compound's mechanism of action probably involves binding to DNA, the DNA binding properties of AMD473 differ from those of cisplatin. First, on naked DNA, several adducts unique to AMD473 were observed (7); second, DNA interstrand cross-links (adducts which some groups have shown to be of importance in determining the antitumor properties of cisplatin) were formed much more slowly in cells exposed to AMD473 compared to cisplatin (peak formation of 5 h for cisplatin *versus* 14-24 h for AMD473) (7); third, a polyclonal antibody raised to DNA adducts of AMD473 showed no cross-reactivity with cisplatin-DNA adducts (9). As with other DNA-damaging agents, induction of p53 was observed in cell lines possessing functional protein although the rate of induction was markedly slower and lasted longer than with cisplatin (8).

Activity against cisplatin-resistant cell lines

As mentioned above, a key preclinical experiment in the drug discovery cascade was an evaluation of each compound's ability to circumvent acquired cisplatin resistance in tumor cell lines. Against 3 pairs (parent and cisplatin-resistant) of human ovarian carcinoma cell lines, 41M/41McisR, CH1/CH1cisR and A2780/A2780cisR, AMD473 showed generally better circumvention of resistance than carboplatin, tetraplatin (a clinically evaluated close analog of oxaliplatin), JM216 or JM118 (the major platinum (II) metabolite of JM216) in all 3 pairs (8) (Table I). In common with the clinical observations, a high degree of cross-resistance was observed between cisplatin and carboplatin. These cell line pairs were selected on the basis of encompassing all of the known major mechanisms of resistance to cisplatin: 41McisR being resistant primarily through reduced drug transport, CH1cisR through enhanced DNA repair/tolerance and A2780cisR through a combination of decreased transport, enhanced DNA repair/tolerance and elevated GSH levels. Platinum transport studies following exposure of the 41M and A2780 pair of lines to AMD473 showed, in contrast to results obtained with cisplatin, equal intracel-

Table I: Circumvention of acquired cisplatin resistance in vitro *by AMD473: comparison with other platinum drugs.*

Drug	Cell Line Pair		
	Resistance Factor (IC_{50} resistant/parent line)		
	41M/41McisR	CH1/CH1cisR	A2780/A2780cisR
Cisplatin	4.7	6.4	16.2
Carboplatin	2.6	4.6	14.3
Tetraplatin	1.8	2.9	4.3
JM216	1.1	4.0	4.5
JM118	0.95	5.7	4.8
AMD473	1.3	2.5	1.9

lular drug levels in the parent and acquired resistant lines (8).

In terms of *in vitro* growth inhibition, AMD473 showed intermediate potency (mean IC_{50} of 8.1 μM) between that of cisplatin (mean IC_{50} of 2.6 μM) and carboplatin (mean IC_{50} of 20.3 μM) across a range of human ovarian carcinoma cell lines. Interestingly, across the National Cancer Institute 60-cell line panel, the COMPARE analysis revealed that AMD473 possesses a distinct pattern of response from all other platinum agents (8).

In vivo *antitumor properties*

As with previous platinum compounds arising from the Cancer Therapeutics/Johnson Matthey collaboration, including carboplatin, the first *in vivo* studies used mice bearing the murine ADJ/PC6 plasmacytoma. Significant antitumor activity was observed following a single intraperitoneal administration of AMD473 (10). A 90% reduction in tumor mass was observed at a dose of 3 mg/kg, whereas severe toxicity was not observed until a dose of 43 mg/kg was used (therapeutic index, TI of 14.3). Moreover, activity was observed against several human ovarian tumors grown as xenografts in nude mice, including some possessing acquired resistance to cisplatin. Activity was also observed against CH1 ovarian xenografts that had regrown following initial treatment with cisplatin (Fig. 1).

In addition, antitumor activity has been observed following oral administration. In mice bearing the ADJ/PC6 plasmacytoma, a therapeutic index of 90 was obtained. Antitumor activity following oral dosing (greater activity than intravenously administered cisplatin or carboplatin and orally administered JM216) was also observed against the acquired cisplatin resistant CH1cisR xenograft (10).

Pharmacokinetics and Metabolism

Platinum pharmacokinetics following i.v. administration to mice showed a biexponential decay in plasma with a rapid distribution ($t_{1/2\alpha}$ of 24 min) followed by a slow elimination ($t_{1/2\beta}$ of 44 h). Following oral dosing, platinum absorption was rapid (tmax of 0.5 h) with a bioavailability of 40% (10). Platinum accumulated mainly in the liver, kidney and spleen. Biotransformation studies involving incubations in human plasma, exposure to tumor cells and dosing to mice have been performed. Parent AMD473 was detectable up to 6 h postadministration (i.p. or oral) to mice. The drug is mainly biotransformed to aquated activation products (11). Notably, and in contrast to results obtained for JM216 (12), only AMD473 itself was detectable within human ovarian carcinoma cells; no GSH adduct was formed (11).

Toxicity

The dose-limiting toxicity of AMD473 in mice and rats is myelosuppression (leukopenia and thrombocytopenia). No renal, liver or neurotoxicity has been observed (10). Thus, the toxicity in rodents is more like carboplatin than cisplatin.

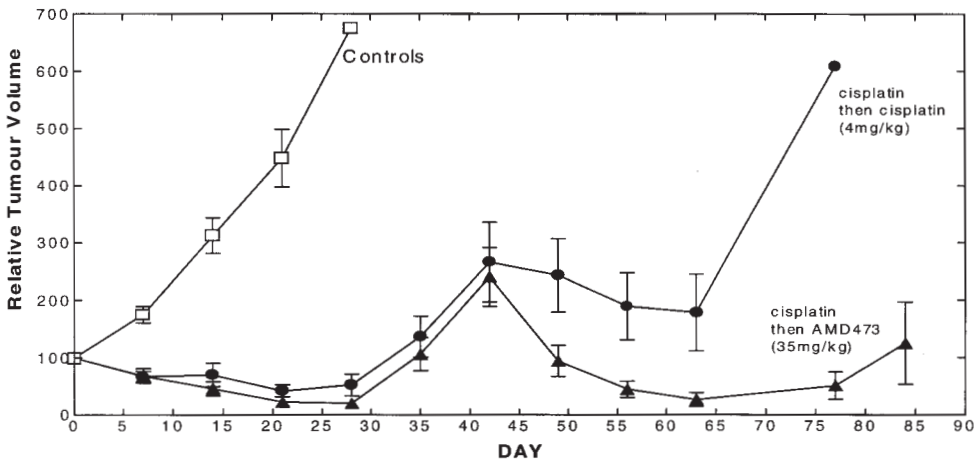


Fig. 1. *In vivo* antitumor activity of AMD473 against CH1 human ovarian carcinoma xenografts at regrowth following treatment with cisplatin. Drug treatments on days 0, 7, 14 and 21; retreatment on days 42, 49 and 56. (Reproduced by permission from Clinical Cancer Research 1997, 3: 2063-74.)

Clinical Studies

In 1996, AMD473 was selected for phase I clinical trials under the auspices of the U.K. Cancer Research Campaign. The initial phase I study, single-dose intravenous administration, began at the Royal Marsden NHS Trust Hospital in November 1997. Subsequently, in April 1998 the drug was licenced to Zeneca for continuing clinical development.

Conclusions

AMD473 has been shown to possess a number of promising preclinical properties: (a) *in vitro* circumvention of acquired cisplatin resistance in cell lines where resistance was due to all of the major known biochemical mechanisms – decreased transport, increased glutathione and enhanced DNA repair; (b) significant *in vivo* antitumor activity by both the intraperitoneal and oral routes against acquired cisplatin resistant human ovarian carcinoma xenografts; (c) toxicological properties in rodents reminiscent of carboplatin with myelosuppression being dose-limiting; (d) less complex metabolism than JM216, the other mixed amine platinum complex in clinical development; (e) reduced reactivity toward sulphur-containing soft nucleophiles relative to cisplatin, including glutathione; (f) unique DNA binding properties compared to cisplatin. Whether these promising preclinical properties will manifest themselves in providing a relatively non-toxic, broader spectrum, orally administered platinum drug for cancer patients will become clearer in the next few years.

Manufacturer

Zeneca Pharmaceuticals (UK).

References

1. Kelland, L.R. *Platinum complexes.* In: Cancer Therapeutics, Experimental & Clinical Agents, B.A. Teicher (Ed.), Humana Press, Totowa, NJ, 1997, 93-112.

2. Kelland, L.R., Abel, G., McKeage, M.J., Jones, M., Goddard, P.M., Valenti, M., Murrer, B.A., Harrap, K.R. *Preclinical antitumor evaluation of bis-acetato-ammine-dichloro cyclohexylamine platinum(IV): An orally active platinum drug.* Cancer Res 1993, 53: 2581-6.

3. McKeage, M.J., Raynaud, F., Ward, J., Berry, C., Odell, D., Kelland, L.R., Murrer, B., Santabarbara, P., Harrap, K.R., Judson, I.R. *Phase I and pharmacokinetic study of an oral platinum complex given daily for 5 days in patients with cancer.* J Clin Oncol 1997, 15: 2691-700.

4. Mistry, P., Kelland, L.R., Abel, G., Sidhur, S., Harrap, K.R. *The relationships between glutathione, glutathione-S-transferase and cytotoxicity of platinum drugs and melphalan in eight human ovarian carcinoma cell lines.* Br J Cancer 1991, 64: 215-20.

5. Dhara, S.C. *A rapid method for the synthesis of cis-[$Pt(NH_3)_2Cl_2$].* Indian J Chem 1970, 8: 193-4.

6. Giandomenico, C.M., Abrams, M.J., Murrer, B.A., Vollano, J.F., Rheinheimer, M.I., Wyer, S.B., Bossard, G.E., Higgins, J.D. III. *Carboxylation of kinetically inert platinum(IV) hydroxy complexes. An entrée into orally active platinum(IV) antitumor agents.* Inorg Chem 1995, 34: 1015-21.

7. Holford, J., Raynaud, F., Murrer, B.A., Grimaldi, K., Hartley, J.A., Abrams, M., Kelland, L.R. *Chemical, biochemical and pharmacological activity of the novel sterically hindered platinum coordination complex, cis-[amminedichloro(2-methylpyridine)] platinum(II) (AMD473).* Anti-Cancer Drug Des 1998, 13: 1-18.

8. Holford, J., Sharp, S.Y., Murrer, B.A., Abrams, M., Kelland, L.R. *In vitro circumvention of cisplatin resistance by the novel sterically hindered platinum complex AMD473.* Br J Cancer 1998, 77: 366-73.

9. Miner, C.B., Raynaud, F.I., Holford, J., Kelland, L.R., Hardcastle, A., Aherne, G.W. *Characterisation of a polyclonal antibody raised to DNA adducts of AMD473.* Br J Cancer 1997, 75 (Suppl. 1): Abst 10.

10. Raynaud, F.I., Boxall, F.E., Goddard, P.M., Valenti, M., Jones, M., Murrer, B.A., Abrams, M., Kelland, L.R. *Cis-amminedichloro(2-methylpyridine)platinum(II) (AMD473), a novel sterically hindered platinum complex: In vivo activity, toxicology, and pharmacokinetics in mice.* Clin Cancer Res 1997, 3: 2063-74.

11. Raynaud, F.I., Boxall, F.E., Wong, T., Goddard, K., Nutley, B., Barnard, C.F.J., Murrer, B., Abrams, M., Kelland, L.R. *Biotransformation of AMD473, a novel platinum analogue in plasma, cell culture and tissue.* Ann Oncol 1998, 9 (Suppl. 2): Abst 190.

12. Raynaud, F.I., Odell, D.E., Kelland, L.R. *Intracellular metabolism of the orally active platinum drug JM216: Influence of glutathione levels.* Br J Cancer 1996, 74: 380-6.