

Advances in Platinum Complex Cancer Chemotherapy

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It is over twenty years since cisplatin was first given to cancer patients, and a decade since the initiation of clinical trials of carboplatin. Nevertheless, platinum-based cancer therapy remains a topic of intensive laboratory and clinical research, as indicated by the presentation of over 300 papers at the Sixth International Symposium on Platinum and Other Metal Co-ordination Compounds in Cancer Chemotherapy, held last year (1). As the clinical trial data on cisplatin and carboplatin have matured their long-term efficacy and toxicities have become evident. The search for new platinum analogues continues and several of these initiatives, encompassing an assortment of chemical structures, have recently reached early Phase clinical trials. Resistance to cisplatin and carboplatin remains the major limitation. The current understanding of the mechanisms of tumour cell resistance and the cellular pharmacology of these agents could lead to new strategies to combat this frustrating problem.

Clinical Trials of Cisplatin and Carboplatin

The platinum derivatives are among the most active agents for the treatment of advanced cancer. Their most dramatic effect has been on the long-term survival of patients with advanced testicular cancer. This is an uncommon tumour-type but the average age of sufferers is only 30 years. Prior to the discovery of cisplatin the cure rate for this tumour was only 5 to 10 per cent. However, with current cisplatin-based chemotherapy protocols approximately 80 to 90 per cent of these patients can expect to survive long-term, free of disease (2). Long-term survival has also been demonstrated in advanced ovarian cancer patients following platinum chemotherapy. Approximately 30 per cent of

patients with Stage III ovarian cancer (that is disease disseminated throughout, but confined to, the abdominal cavity) will live for at least 10 years following cisplatin-based therapy, whereas, without the use of cisplatin the survival rate is only about 10 per cent (3). Recent data, well reviewed by Smith and Talbot (4), has also suggested a possible role for cisplatin in the treatment of advanced breast cancer. This disease is responsible for approximately 12,000 deaths each year in the U.K. alone. Originally, interest in cisplatin was not great because studies in patients with refractory breast cancer had shown the drug to have little activity. Latterly, studies of cisplatin in previously untreated patients have shown activity comparable to some of the best available conventional regimens for this disease, for example, cyclophosphamide, methotrexate and 5-fluorouracil (CMF). Cisplatin has been combined, for the treatment of this tumour, with agents such as etoposide which show synergism with the platinum complex in other tumour types.

In spite of its impressive anti-tumour activity, treatment with cisplatin does result in severe toxicity. The most immediate and often the most disabling toxicities are nausea and vomiting (emesis). Use of corticosteroids, high dose metoclopramide, antihistamines and benzodiazepines can improve symptoms in some patients, but often at the expense of side-effects such as drowsiness and involuntary movements (referred to as dystonic movements). Emesis with cisplatin has been shown to be stimulated by the binding of 5-hydroxytryptamine (5-HT) to the 5-HT₃ receptor. Inhibitors of this receptor, such as ondansetron, have resulted in the dramatic alleviation of nausea and vomiting without the side-effects induced by other anti-emetic treatments (5). Furthermore, the

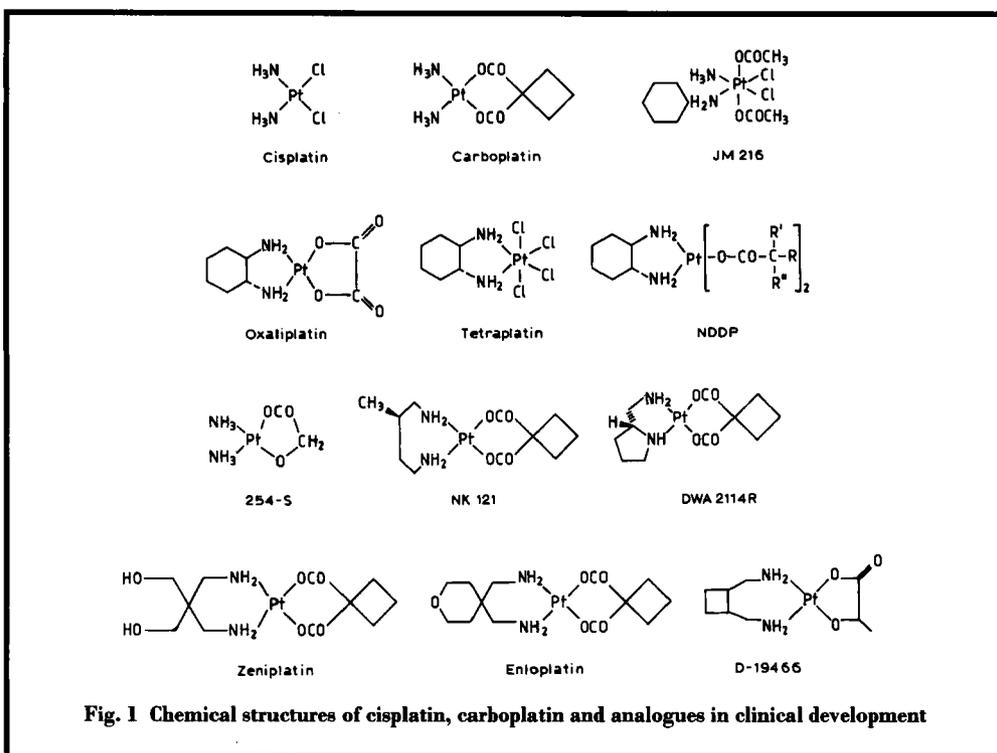


Fig. 1 Chemical structures of cisplatin, carboplatin and analogues in clinical development

combination of a 5-HT₃ inhibitor with dexamethasone has been shown to reduce significantly the number of vomiting episodes in the first 24 to 48 hours after cisplatin-containing combinations by 78 per cent, as compared with 30 per cent with ondansetron alone (6). Nerve damage is now the major dose-limiting factor for cisplatin since acute kidney damage has been substantially mitigated by the use of intravenous hydration. The predominant neurotoxic effect is the loss of sensory function in the limbs (peripheral neuropathy), but disturbances of hearing, balance and vision can also occur. Interestingly, workers from The Netherlands have identified a neuroprotective peptide (Org 2766) which, in randomised clinical trials, has been demonstrated to prevent the development of peripheral neuropathy in patients receiving cisplatin treatment for ovarian cancer (7). The long-term survival of patients following treatment has provided the opportunity to document the incidence of chronic side-effects from cisplatin. These are substantial and variously

include: permanently impaired kidney function, peripheral neuropathy, impaired hearing, Raynaud's phenomenon (a disorder causing symptoms like chilblains), psycho-sexual difficulties, impairment of sex hormone production, elevated blood pressure and elevations in blood cholesterol (8).

The severe toxicities of cisplatin are, to a large extent, circumvented by the use of carboplatin. This analogue causes little in the way of kidney or nerve damage, and the nausea and vomiting is less intense than with cisplatin. Randomised comparative trials of these two agents in both ovarian (9) and lung cancer (10) have confirmed their therapeutic equivalency, as well as substantiating the clinical advantages for carboplatin in terms of reductions in the severity and incidence of side-effects, and improved patient acceptability (11). Furthermore, it is now possible to give platinum chemotherapy in the course of a short visit to the outpatient's department, since intravenous hydration and high-dose anti-emetics are generally not required

with carboplatin. Bone marrow suppression is the major toxicity of carboplatin, with the predominant consequence being a transient reduction in the peripheral blood platelet count. The individualisation of carboplatin dosages according to kidney function using a formula first developed by Calvert (12) has made bone marrow suppression highly predictable and more manageable. Initiatives to circumvent bone marrow toxicity, such as autologous bone marrow transplantation and colony stimulating factors, are being studied in an attempt to increase both the dose and therapeutic activity of carboplatin (13). Such approaches rely on the existence of a steep relationship between the dose of drug and response of the tumour. A recent retrospective analysis of the dose-response relationship for carboplatin in ovarian cancer, however, suggests that this relationship is rather flat above conventional doses (14).

New Cisplatin Analogues

The chemical structures of ten cisplatin analogues currently undergoing early phase clinical testing are shown in Figure 1. These complexes include (i) diaminocyclohexane derivatives, (ii) platinum(II) complexes with dicarboxylate cyclobutane or related oxygenated leaving groups, analogous to carboplatin, and, (iii) an orally active mixed ammine/amine platinum(IV) complex. We have recently written a comprehensive review of the current status of these clinical trials to which the reader is referred for further details and references (15).

The rationale for the development of diaminocyclohexane (DACH) platinum complexes has been the property of non-cross resistance in cisplatin-resistant murine leukaemias. However, considerable doubt now exists as to the utility of the murine leukaemias as a model of cisplatin-refractory disease, since DACH compounds are frequently cross-resistant with cisplatin in alternative models of platinum resistance, for example, human ovarian carcinoma xenografts (16). A total of eleven DACH complexes have entered clinical trials over the last two decades, but most of these failed early in development because of either formulation dif-

iculties or unacceptable clinical toxicity. Three DACH compounds remain in clinical trials.

Tetraplatin is a racemic mixture of the *l-trans*- and *d-trans*- isomers of tetrachloro(1,2-diaminocyclohexane)platinum(IV). This is the second platinum(IV) complex to enter clinical trials, the first being iproplatin. Phase I dose-finding studies are currently underway in the United States of America, and the toxicities encountered in these trials so far include nausea and vomiting, bone marrow suppression, mild liver damage and cumulative damage to the peripheral nervous system. Oxaliplatin, the *trans-l*-isomer of oxalato-1,2-diaminocyclohexaneplatinum(II), is in clinical trials in France. In Phase I studies the dose-limiting toxicity was nerve damage, which was unusual in that it was characterised by the acute onset of paraesthesia (a sensation of pins and needles) during the drug infusion and the development of the cumulative loss of sensation in limbs (peripheral neuropathy) with repeated dosing. Phase II trials are ongoing and at this time oxaliplatin's single agent activity and utility in platinum refractory disease are unknown. The third DACH complex in the clinic comprises of a racemic mixture of *cis*-bis-neodecanoato-*trans-R,R*-1,2-diaminocyclohexane platinum (II) (L-NDDP) formulated within spherical lipid drug carriers (liposomes). Phase I studies of L-NDDP, given both intravenously and directly into the artery supplying the liver, have been conducted at the M.D. Anderson Cancer Centre (Houston, Texas). This preparation seems well tolerated, with blood count suppression being the major side-effect. Interestingly, little nerve damage has been seen. Laboratory studies are now focusing on the biological properties of the NDDP isomers and optimal constituents of the liposomal component.

As shown in Figure 1, several of the platinum complexes currently in early phase clinical trials have either dicarboxylate cyclobutane or closely related oxygenated leaving groups, conferring good water solubility and stability by comparison to cisplatin. In these respects these compounds are analogous to carboplatin. Both enloplatin, [1,1-cyclobutane-dicarb-

oxylato-(2-)-O,O] [tetrahydro-4H-pyran-4,4-dimethanamine-N,N] platinum(II) (CL287, 110), and zeniplatin, [2,2-bis (aminomethyl)-1,3-propanediol-N,N'] [1,2-cyclobutane-dicarboxylato(2-)-O,O'] platinum(II) (CL286,558) are in development by the American Cyanamid Company. Both have identical leaving groups to carboplatin. However, unlike carboplatin, both zeniplatin and enloplatin caused kidney toxicity at maximally tolerable doses during Phase I trials. Interestingly, this finding appears contrary to the proposed relationship between leaving group stability and the nephrotoxicity of platinum complexes. Japanese efforts have focused on three analogues: (i) diammine (glycolato-O,O')platinum(II) (254-S), (ii) (R)-2-aminomethyl-pyrrolidine(1,1-cyclobutane-dicarboxylato)platinum(II) monohydrate (DWA 2114R), and (iii) *cis*-1,1- cyclobutane-dicarboxylato(2R)-2-methyl-1,4- butanediamine platinum (II) (NK-121, CI-973). The clinical properties of these compounds are largely similar to carboplatin, with the exception of more pronounced gut toxicity with protracted administration schedules of DWA2114R, and white blood cell count depression rather than platelet count depression being dose-limiting for NK121. Finally, Phase I studies of a platinum(II) complex (1,2-diaminomethyl-cyclobutane-platinum(II)-lactate (Lobaplatin, D19466) under development by ASTA-Medica (Frankfurt, Germany) have recently been reported. In these studies thrombocytopenia, leucopenia, emesis and objective tumour responses were recorded.

Carboplatin is now clearly established as superior to cisplatin in terms of patient compliance, and the severity and incidence of side-effects (11). However, both cisplatin and carboplatin are given intravenously. The compliance and quality of life of cancer patients receiving platinum-based chemotherapy could, potentially at least, be further enhanced by the development of an oral platinum preparation. To this end, early phase clinical trials of an orally administrable platinum complex (ammine diacetato dichloro (cyclohexylamine) platinum(IV) (JM216), see Figure 1, have recently started

at the Royal Marsden Hospital (Sutton, U.K.). This agent is the product of a collaboration between workers at the Institute of Cancer Research (Sutton, U.K.), The Johnson Matthey Technology Centres (Sunning Common, U.K.; West Chester, Pennsylvania, U.S.A) and Bristol-Myers Squibb Oncology Division (Wallingford, Connecticut, U.S.A.). Preclinical work has shown that after oral administration, JM216 has comparable activity to cisplatin and carboplatin given intravenously in a panel of four human ovarian carcinoma xenografts *in vivo*. Additionally, in rodents, oral JM216 has shown a lack of damage to the kidneys. The dose-limiting toxicity in mice was depression of peripheral blood white cell counts. Therefore, this platinum(IV) anti-tumour complex is well absorbed from the gastrointestinal tract and has toxic effects comparable to carboplatin.

In short, both neurotoxicity and lack of activity in human tumour models could be major limitations for the DACH complexes, while any clinical advantages for the "carboplatin analogues" over carboplatin itself are unclear at the present time. Finally, the successful development of an oral platinum drug could facilitate both the administration of out-patient chemotherapy and clinical studies on the schedule-dependency of platinum therapy.

How Do Tumour Cells Become Resistant to Platinum Drugs?

It is a well established clinical phenomenon that some patients whose tumours initially respond well to platinum-based chemotherapy become refractory to subsequent treatments. An understanding at the biochemical and molecular level of how such resistance develops might lead to the rational design of a new generation of more effective platinum-based anti-cancer drugs.

In a further collaborative venture between biologists at our Institute, chemists at the Johnson Matthey Technology Centres, and Bristol-Myers Squibb Oncology Division we are seeking to discover new platinum-containing anti-cancer agents with a broader spectrum

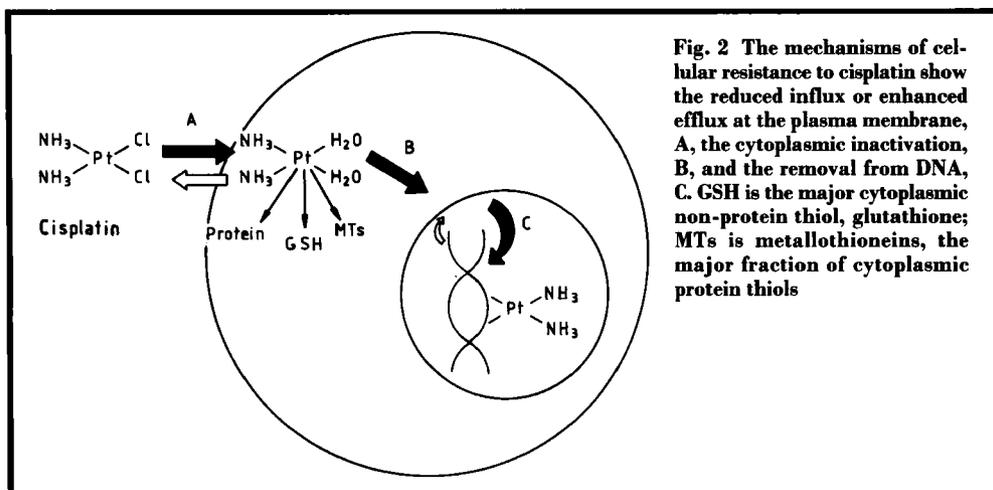


Fig. 2 The mechanisms of cellular resistance to cisplatin show the reduced influx or enhanced efflux at the plasma membrane, A, the cytoplasmic inactivation, B, and the removal from DNA, C. GSH is the major cytoplasmic non-protein thiol, glutathione; MTs is metallothioneins, the major fraction of cytoplasmic protein thiols

of activity than the currently available drugs. At the biochemical level, our efforts have concentrated on the development of appropriate laboratory models of cisplatin resistance and the elucidation of the mechanisms underlying their resistance. Using tissue culture methodology, we have grown tumour cells from patients presenting with advanced ovarian cancer representative of both responding tumours and those refractory to platinum-based chemotherapy (17). In addition, resistance to cisplatin has been generated in the laboratory by exposing tumour cells to cisplatin *in vitro* over several months (18).

A pictorial view of cisplatin being delivered to a tumour cell is shown in Figure 2. For the platinum to reach the DNA within the nucleus of the tumour cell, where it binds to produce its cell killing effects, it first has to traverse the cell membrane and then pass through the cytoplasm. There is experimental evidence indicating that resistance may occur at each of these three levels, as shown in Figure 2, that is, **A** = reduced influx or enhanced efflux at the plasma membrane, **B** = cytoplasmic inactivation, **C** = removal from DNA. Indeed, with some cisplatin-resistant tumours, resistance appears to have occurred at all three levels; for a review see (19).

Resistance mediated at the level of the plasma membrane is a common feature of cisplatin-

resistant tumour cells. This occurs mainly through reduced drug influx rather than the increased pumping out of drug observed for some other commonly used anti-cancer drugs, for example, adriamycin, etoposide and vincristine. While it is still not entirely clear how cisplatin enters cells, whether by passive diffusion and/or active transport, in recent years changes in some membrane proteins have been associated with resistance to cisplatin (20, 21).

Cisplatin and carboplatin react avidly with sulphur ligands. Both the major cytoplasmic non-protein thiol, glutathione (GSH), and the major fraction of cytoplasmic protein thiols, metallothioneins (MTs), have been shown to be elevated in some cisplatin-resistant cells. Platinum binds to these increased levels of intracellular thiols to form inactive species, labelled **B** in Figure 2, thereby preventing the active aquated species from reaching the nucleus.

Once the platinum has successfully by-passed the resistance mechanisms occurring at the plasma membrane and in the cytoplasm, the tumour cell is still able to resist the potentially lethal effects of the drug at the level of DNA itself, labelled **C** in Figure 2. Platinum forms covalent adducts on guanine, and to a lesser extent adenine bases, on DNA. Approximately ninety-eight per cent of the adducts are formed on the same strand of DNA and are termed intrastrand crosslinks. Such a lesion is shown in Figure 2.

The remainder are formed between the two DNA strands and are termed interstrand crosslinks; for a review see (22). If these platinum-containing adducts on DNA are not removed the cell may not be able to divide successfully and such cells will ultimately die. Both normal and tumour cells, however, possess numerous enzymes which work together in specific pathways to remove such adducts from their DNA (22). It is clear that at least some cisplatin-resistant tumour cells possess an enhanced capacity to remove platinum-induced adducts from their DNA and thereby have the capacity to by-pass the potentially lethal effects of the drug.

Our collaborative programme is aimed at developing new platinum-containing complexes to tackle the above mechanisms of tumour cell resistance. As both cisplatin and carboplatin possess diammine ligands, which are retained as part of the DNA-platinum adduct, one strategy has been to synthesise complexes with asymmetric ammine/amine (termed "mixed amine") ligands. Such mixed amine complexes, of which the orally active platinum(IV) dicarboxylate,

JM216, is an example, may produce a different spectrum of adducts on DNA and be subject to differing removal pathways than either cisplatin or carboplatin. Hence, they may at least partially overcome resistance which is due to enhanced removal of adducts from DNA. Furthermore, some of the ammine/amine platinum(IV) dicarboxylates in which the axial chain (R1) has been extended to greater than three carbons have been shown to be over 100-fold more potent than cisplatin against some cisplatin-resistant human ovarian tumour cells (23). Such agents, which are considerably more lipophilic than cisplatin, appear to be particularly effective at overcoming cisplatin resistance which is due to reduced uptake (18).

We are hopeful that these exciting laboratory-based in vitro findings will also eventually be achievable in cancer patients and lead to a new generation of even more effective platinum-based anti-cancer drugs.

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Ruthenium and Palladium in Hydrogen Detection

DOPED LEAD PHTHALOCYANINE WITH HIGH SENSITIVITY

In recent years metal phthalocyanines have been investigated for use as host materials in gas sensors. These organics are *p*-type semiconductors with very high resistivity, and sensors made from them are generally only suitable for detecting oxidising gases, including nitrogen dioxide and chlorine.

Now researchers at the College of Industrial Technology, Nihon University, Japan, have reported the results of a study made to overcome the difficulties that may be associated with the use of organic semiconductors as sensors for reducing gases such as hydrogen and carbon monoxide ("The Detection of H₂ Gas by Metal Phthalocyanine-Based Gas Sensors", S. Kanefusa and M. Nitta, *Sens. Actuators B*, 1992, 9, (2), 85-90).

Sensors were fabricated on high-purity alumina substrates printed with gold electrodes. Cobalt, lead, magnesium, nickel and zinc phthalocyanines were tested and sensors based on lead phthalocyanine were found to exhibit the highest sensitivity to hydrogen gas. Adding palladium black to the phthalocyanine increased its sensitivity and responsivity, by catalytic reaction. It also decreased the resistance, which was lowered still further by adding ruthenium oxide to the lead phthalocyanine and by building up the thickness of the sensor film to about 40µm.

The sensitivity of the sensors was found to be dependent on both the additions and the operating temperature, increasing with increasing sensor temperature and reaching a maximum at about 160°C. The highest sensitivity was exhibited by sensors doped with 10 weight per cent ruthenium oxide and one weight per cent palladium. For a sensor doped with 10 per cent ruthenium oxide and two per cent

palladium the highest sensitivity and hydrogen response occurred at 120°C.

When the hydrogen concentration was 8000 ppm, the resistivity of lead phthalocyanine doped with ruthenium oxide and palladium was 10 times lower than it was in air, at 120°C.

Although pure lead phthalocyanine behaves as a *p*-type semiconductor, doping it with ruthenium oxide and palladium changes its semiconductor properties to that of an *n*-type semiconductor. This change in behaviour is considered.

The lead phthalocyanine sensors doped with ruthenium and palladium can be used at lower temperatures than ceramic sensors, but in practice they should be used above 100°C as atmospheric humidity can disturb the sensitivity at lower temperatures.

Towards a Viable Fuel Cell

Fuel cells to be used for traction purposes would advantageously be powered by a liquid fuel supplied from the existing oil distribution network; the properties of methanol make it attractive for this purpose. Interestingly, the only effective catalysts for the electro-oxidation of methanol are based upon platinum, although the mechanism of this reaction remains controversial.

Ways of increasing the effectiveness of platinum based catalysts for methanol oxidation are considered in a recent paper (A. Hamnett and G. L. Troughton, *Chem. Ind. (London)*, 1992, (13), 480-483). Superior catalysts based on ternary alloys, better ways of using existing solid proton-conducting membranes and new membrane materials may all contribute to the development of a commercially viable fuel cell.