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Cisplatin and platinum drugs at the molecular level (Review)

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Abstract. Over twenty years of intensive work toward improvement of cisplatin, and with hundreds of platinum drugs tested, has resulted in the introduction of the widely used carboplatin and of oxaliplatin used only for a very narrow spectrum of cancers. A number of interesting platinum compounds including the orally administered platinum drug JM216, nedaplatin, the sterically hindered platinum (II) complex ZD0473, the trinuclear platinum complex BBR3464, and the liposomal forms Lipoplatin and SPI-77 are under clinical evaluation. This review summarizes the molecular mechanisms of platinum compounds for DNA damage, DNA repair and induction of apoptosis via activation or modulation of signaling pathways and explores the basis of platinum resistance. Cisplatin, carboplatin, oxaliplatin and most other platinum compounds induce damage to tumors via induction of apoptosis; this is mediated by activation of signal transduction leading to the death receptor mechanisms as well as mitochondrial pathways. Apoptosis is responsible for the characteristic nephrotoxicity, ototoxicity and most other toxicities of the drugs. The major limitation in the clinical applications of cisplatin has been the development of cisplatin resistance by tumors. Mechanisms explaining cisplatin resistance include the reduction in cisplatin accumulation inside cancer cells because of barriers across the cell membrane, the faster repair of cisplatin adducts, the modulation of apoptotic pathways in various cells, the upregulation in transcription factors, the loss of p53 and other protein functions and a higher concentration of glutathione and metallothioneins in some type of tumors. A number of experimental strategies to overcome cisplatin resistance are at the preclinical or clinical level such as introduction of the bax gene, inhibition of the JNK pathway,

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introduction of a functional p53 gene, treatment of tumors with aldose reductase inhibitors and others. Particularly important are combinations of platinum drug treatments with other drugs, radiation and the emerging gene therapy regimens.

Contents

1. Introduction

The most prevalent problem in cancer therapy is the regrowth and metastasis of malignant cells after standard treatment with

Figure 1. The structure of cisplatin (left) and transplatin (right)

surgery, radiation, and/or chemotherapy. Additional hurdles arise from chemoresistance of tumors, toxicity of currently available chemotherapy regimens and inefficiency of cancer treatments especially for advanced stage of the disease. The advent of taxanes as a standard treatment for major cancers, including breast and lung cancers, has brought a revolution in cancer chemotherapy although the increase in life extension is rather small.

Cisplatin, or cis-diaminodichloro-platinum (II), (Fig. 1) lies at the intersection of many fields of study. It is not only a cornerstone in the development of present day co-ordination theory, but also one of the most effective and potent anticancer drugs. First synthesized by M. Peyrone in 1847, cisplatin was part of the co-ordination theory revolution of Alfred Werner in 1893. In the 1960's Barnett Rosenberg serendipitously discovered its chemotherapeutic cancer activity. Initial clinical tests by Hill *et al* in 1971, showed cisplatin to be active against malignant lymphoma, Hodgkin's disease, and certain other malignancies (reviewed in refs. 1,2). Its stereoisomer, transplatin, is not endowed with the anticancer activity of cisplatin.

Cisplatin is one of the most widely used and most effective cytotoxic agents in the treatment of epithelial malignancies such as lung, head-and-neck, ovarian, bladder and testicular cancer (3,4). However, its continued clinical use is impeded by its severe adverse reactions including renal toxicity from renal tubular damage, gastrointestinal toxicity, peripheral neuropathy, asthenia, and ototoxicity (1,2). The ototoxicity arises from apoptosis in auditory sensory cells induced by cisplatin (5). The cumulative dose of cisplatin is a strong risk factor for the development of nephrotoxicity in patients who receive high doses of ifosfamide, cisplatin and etoposide combinations (6). Bilateral optic neuropathy was observed in a patient affected by ovarian carcinoma treated with 160 mg/m^2 cisplatin and 640 mg/m^2 carboplatin (7).

This article reviews the basic mechanisms involving cisplatin and other platinum drugs and explores the basis of platinum resistance.

2. Interactions of cisplatin with DNA

The mode of action of cisplatin is still not completely understood but it is thought to depend on hydrolysis reactions where the –Cl group is replaced by a water molecule adding a positive charge on the molecule. The hydrolysis product is believed to be the active species reacting mainly with glutathione in the cytoplasm and the DNA in the nucleus thus inhibiting replication, transcription and other nuclear functions and arresting cancer cell proliferation and tumor growth. A number of additional properties of cisplatin are now emerging including activation of signal transduction pathways leading to apoptosis. Firing of such pathways may originate at the level of the cell membrane after damage of receptor or lipid molecules by cisplatin, in the cytoplasm by modulation of proteins via interaction of their thiol groups with cisplatin, for example involving kinases, and other enzymes or finally from DNA damage via activation of the DNA repair pathways.

Cisplatin reacts with nucleophilic sites in DNA forming monoadducts as well as intra- and interstrand crosslinks. In general, cisplatin crosslinks can be on the same strand of DNA (Fig. 2a), or on adjacent points between two strands (Fig. 2b). The major product is 1,2-interstrand d(GpG) crosslinks and secondarily d(ApG) crosslinks. Cisplatin can form monoadducts on the DNA (Fig. 2c) with the other reactive group still able to interact with reactive thiols, amino, hydroxyl or other groups. Finally cisplatin can form monoadducts on the DNA but simultaneously mediate crosslinking of a bulky protein or a small molecule, such as glutathione, to DNA (Fig. 2d).

The preferred site of cisplatin DNA binding was in the linker region of the nucleosome. Carboplatin and other platinum compounds including an acridine-platinum complex were also found to target the linker region of the nucleosome (8). The crucial steps in the antitumor activity of analogs of cisplatin that contain chiral amine ligands are at the interaction with DNA and formation of crosslinks with adjacent purine bases. DNA crosslinks of platinum complexes with enantiomeric amine ligands not only can exhibit different conformational features but also can be processed differently by the cellular machinery (9).

3. Interactions of cisplatin with thiol groups and macromolecules

In addition to DNA, amino acids, peptides, proteins (for example metallothioneins), and small molecules (glutathione) are very much involved in the metabolism of cisplatin. The sulfur-containing tripeptide glutathione (GSH) is one of the most abundant SH-containing molecules in cells. Platinum complexes are often very reactive towards the cysteine residue of GSH, which detoxifies these compounds by a rapid binding mechanism (10).

Cancer cells have different levels of glutathione and metallothionein that can detoxify platinum drugs intracellularly or extracellularly by interaction with their SH groups preventing them from binding to DNA. Elevated levels of glutathione render some types of cancer cells resistant against cisplatin and carboplatin. For example, the cisplatin-resistant cell line A2780cisR, possesses elevated levels of glutathione. This mechanism of interaction of platinum drugs with glutathione poses a severe obstacle to new platinum drugs designed to overcome cisplatin resistance (11).

The plasma membrane enzyme gamma-glutamyl transpeptidase (GGT), plays a crucial role in catabolism of extracellular glutathione and cellular handling of thiols; a balance between transport of sulfur amino acids and GGT activity results in profound differences in the capability of cells to modify the thiol redox status of the extracellular milieu. GGT is often expressed in malignant tumors, including melanomas, although its expression levels may vary widely among different tumors or cells of the same tumor. Human clones can be established from tumor specimens with high or low GGT activity. GGT-rich cells
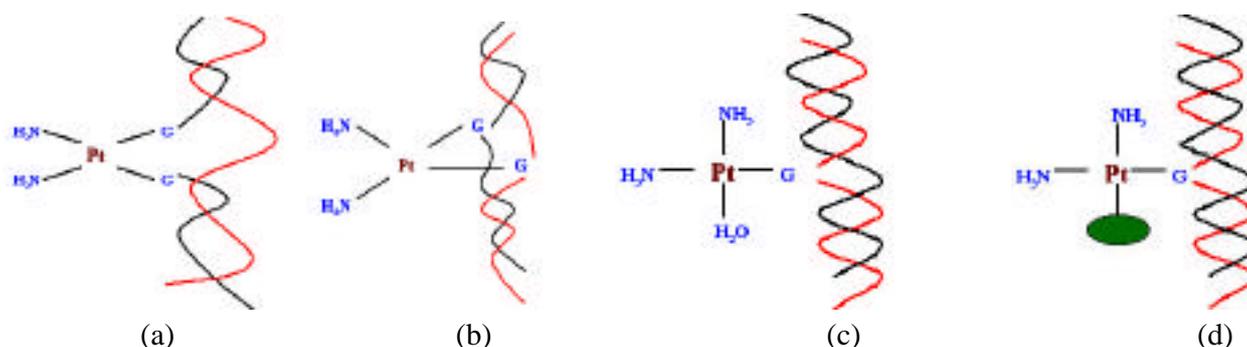
Figure 2. Various products formed from cisplatin and DNA: (a) intrastrand crosslinks; (b) interstrand crosslinks; (c) monoadducts with one reactive group (shown here in its hydroxo form) on the monoadduct still available for reaction; (d) monoadducts with the other reactive group linked to a small molecule (glutathione) or to a nuclear protein.

had lower intracellular glutathione levels whereas GGT-poor cells exhibited extracellular accumulation of glutathione (GSH), GSSG and glutathione-cysteine disulfide. Clones with high GGT activity were more sensitive to platinum compounds (12).

4. Repair of cisplatin lesions

Cisplatin adducts are repaired by the nucleotide excision repair pathway (NER). The first step for NER is recognition of DNA damage. The DNA mismatch repair genes and hMSH2 in combination with one of its heterodimer partners binds specifically to cisplatin adducts (13).

Defects in DNA mismatch repair, resulting from mutation or methylation-mediated silencing of hMLH1, hMSH2, or hPMS2, produce low level resistance to cisplatin that seems to be caused by a failure to recognize the adduct and propagate a signal to the apoptotic machinery. The proteasome inhibitor, N-acetyl leucyl-leucyl norlucinal (ALLnL) that inhibits the post-translational ubiquitination of histone H2A increased cellular sensitivity to cisplatin in an additive manner in three human ovarian tumor cell lines. This mechanism was exerted by reducing the rate of cisplatin efflux by about 50% and by increasing DNA damage levels from a reduction in DNA repair promoted by ubiquitinated H2A (14).

A significant effort has been devoted to identify proteins able to bind specifically to cisplatin-induced lesions. DNA-cisplatin adducts are specifically recognized by two classes of proteins. One class of proteins recognize DNA damage as an initial step of the nucleotide excision repair and mismatch repair pathways. The other class contains proteins stabilizing cellular DNA-protein and protein-protein complexes, including non-histone proteins from the HMG (high-mobility-group) family. HMG-1 interacts only with the cis- but not with the trans-platin *in vitro* (Fig. 1), a fact that might explain why transplatin is of no therapeutic value. HMG-1 is a nuclear matrix protein interacting with single-strand DNA loops in cruciforms and competing with histone H1 for binding to nucleosomes to maintain an altered, transcription-poised nucleosome conformation. HMG-1 specifically recognizes 1,2-interstrand d(GpG) and d(ApG) crosslinks of DNA-cisplatin adducts and inhibits their repair. Other proteins have been identified, including Ixr1 containing two consecutive HMG boxes, and the

structure-specific recognition protein 1 (SSRP1). SSRP1 recognizes the stem-loop structures, bends, and locally unwound DNA postulated to be formed during recombination of immunoglobulin genes. Transcription factors containing the HMG box, such as hUBF, are also able to bind to DNA sequences containing cisplatin-induced lesions even though the DNA sequence itself does not conform to their cognate binding site; this phenomenon is being referred to as "transcription factor hijacking". XPE involved in the recognition of UV-irradiated DNA also recognizes undamaged single-stranded DNA and cisplatin-damaged DNA (reviewed in 15).

5. Signal transduction, gene expression changes and apoptosis with cisplatin

The major mechanism of cisplatin-induced damage to tumors is via induction of apoptosis (16). Mismatch repair processing is one of the several factors involved in induction of apoptosis (17,18). Activation of proteases can play an important role in apoptotic cell death induced by anticancer drugs; inhibitors of cysteine and serine proteases prevented the cytotoxic effect of cisplatin (19). Ceramide, generated from a distinct subcellular pool of sphingomyelin by the action of sphingomyelinases, may be used by cells to propagate apoptotic signals in response to a variety of cytotoxic agents (20). All trans retinoic acid enhances cisplatin-induced apoptosis and potentiates cisplatin cytotoxicity in ovarian carcinoma cell lines via modulation of the activity of cyclin-dependent kinase2 (CDK2)/cyclin A (21). Carboplatin also activates apoptotic pathways in retinoblastoma cell cultures, increases the level of p53 and p21, and lowers the level of Bcl-2 (22). Suppression of hyperthermia-induced accumulation and activation of p53 by cisplatin or X-rays was cell type specific and it was observed in A-172 but not in T98G human glioblastoma cells (23). ERCC-1 mRNA expression was induced by cisplatin through a DNA damage-response pathway (24); ERCC-1 is one of the essential proteins in nucleotide excision repair participating in cisplatin adduct repair. Introduction of the bax gene as a complex with a cationic lipopolyamine in a human gastric cancer xenograft enhanced the sensitivity of the tumor to the combination of 5-fluorouracil and cisplatin via induction of apoptosis associated with the constitutive overexpression of the bax gene (25).

Apoptosis induction appears to be mediated by activation of signal transduction. Cisplatin is an activator of stress-signaling pathways especially of the c-Jun N-terminal kinase (JNK) family of mitogen-activated protein (MAP) kinases in a variety of cells including small-cell lung cancer (SCLC) cells. Therefore, inhibition of the JNK pathway is a potential means to enhance the sensitivity of SCLC cells to platinum compounds (26,27). Apoptosis in auditory sensory cells induced by cisplatin involved both the death receptor mechanisms as well as mitochondrial pathways (5).

Activation of the mitogen-activated protein kinases ERK1/2 by cisplatin has been shown to result in either survival or cell death; cisplatin-induced activation of ERK was mediated by Ras in Saos-2 osteosarcoma cells. Incubation of these cell lines with the MEK1 inhibitors PD98059 or UO126 after, but not during, cisplatin treatment completely inhibited cisplatin-induced activation of ERK. ERK activation increased cisplatin-induced cell death independently of p53 in osteosarcoma and neuroblastoma cell lines (28). Similar data supporting an important role of ERK in cisplatin-induced apoptosis were obtained by Lee and co-workers (29). Treatment of HeLa cervical carcinoma cells with the aldose reductase inhibitor, ethyl 1-benzyl-3-hydroxy-2(5H)-oxopyrrole-4-carboxylate (EBPC), enhanced the cytotoxic effects of doxorubicin and cisplatin; treatment of HeLa cells with EBPC in combination with doxorubicin or cisplatin increased the extracellular signal-regulated kinase (ERK) activity as compared to treatment with the chemotherapeutic drugs alone, suggesting a possible role for the ERK pathway in mediating doxorubicin- or cisplatin-induced cell death. Inhibition of ERK activation by the MEK inhibitor, U0126, reversed the EBPC-mediated enhancement of cell death. Thus, aldose reductase inhibitors, such as EBPC, may have a place for adjuvant therapy to improve the effectiveness of doxorubicin or cisplatin (29). Phosphorylation of the serine/threonine kinase Akt, a target molecule activated by Src kinase, is induced by cisplatin (30).

Cisplatin induces oxidative stress. Induction of heme oxygenase-1 may serve as an immediate protective response after treatment with cisplatin. Induction of heme oxygenase by hemin decreases damage to a following cisplatin treatment of cell cultures. Oxidative pathways participate in the characteristic nephrotoxicity of cisplatin and the antioxidants alpha-tocopherol and N-acetylcysteine can protect cells from damage (31). A key event in cervical carcinogenesis is the disruption of p53 tumor suppressor pathway by human papillomavirus E6 oncogene. Cisplatin, carboplatin, and oxaliplatin activated a p53 reporter gene and reduced the HPV E6 mRNA. Treatment with platinum drugs after irradiation of cell cultures led to poly(ADP-ribose) polymerase cleavage as a sign of caspase-3 activation and apoptosis (32).

6. Resistance to cisplatin and platinum compounds

The major limitation in the clinical applications of cisplatin has been the development of cisplatin resistance by tumors. This arises either by clonal expansion of tumor cells in the heterogenous tumor cell population with inherent resistance to cisplatin (with mutations in specific genes that confer resistance) or by acquired resistance by some cells in the tumor during treatment and their clonal expansion after killing of the sensitive

cells by the drug. The mutagenic effect of cisplatin would favor the development of mutations in various genes and the selection of cells with proliferation advantage in patients that undergo cisplatin chemotherapy. This proliferation advantage could be conferred by limited uptake of the drug by resistant cells and absence of damage-induced apoptosis or by an enhanced repair of the DNA lesions and signaling in favor of cell survival rather than apoptosis. This leads to an intriguing question for the medical oncologist: Shall I treat chemotherapy-naive patients with cisplatin every 3 weeks, which appears to be the gold standard regimen, or at lower more frequent or even daily doses? Both regimens have been used in clinical trials and the debate is not over (reviewed in ref. 33). Maintenance of cisplatin levels in sera and tumors for prolonged periods of time is expected to eradicate cisplatin-sensitive cells without offering them a chance to develop resistance; then resistance would arise from the cell population with intrinsic resistance to cisplatin. Since also there is cross-resistance to other chemotherapy drugs, first line chemotherapy and its administration schedule are of paramount importance for a successful outcome. Hurdles in any case are the side effects of the drug and the toxicity from the cumulative dose. The probability of response to second line chemotherapy following platinum-based treatments is usually related to the platinum-free interval. Salvage monochemotherapy is generally used, but when the platinum-free interval is longer than 24 months, re-treatment with platinum compounds and/or taxanes is indicated.

Several mechanisms can contribute to cisplatin resistance. The reduction in cisplatin accumulation inside cancer cells because of barriers across the cell membrane is considered a major mechanism of the acquired cisplatin resistance (34,35). An independent mechanism of cisplatin resistance arises from a faster repair of cisplatin adducts. A number of additional mechanisms determine cisplatin resistance to a smaller extent such as induction of different apoptotic pathways in various cells and upregulation in transcription factors (see below).

The copper transporter CTR1 appears to control the accumulation of cisplatin in Saccharomyces cerevisiae. Deletion of CTR1 resulted in a 16-fold reduction in the uptake of copper and an 8-fold reduction in the uptake of cisplatin. CTR1-deficient cells were 1.9-fold more resistant to the cytotoxic effect of cisplatin than the CTR1-replete cells. CTR1-deficient cells also demonstrated impaired accumulation of the cisplatin analogs carboplatin, oxaliplatin, and ZD0473 (36).

The homeobox gene BARX2 may be a biological factor involved in determining sensitivity or resistance to the cytotoxic effects of cisplatin in epithelial ovarian cancer (37). Suppression of the multidrug resistance-associated protein (MRP) and multidrug resistance 1 (MDR1) gene expression in HCT-8DDP human colon cancer cell lines using hammerhead ribozymes, designed to cleave the MRP and MDR1 mRNAs, was sufficient to reverse multidrug resistance to doxorubicin and etoposide (VP-16) but did not affect resistance to cisplatin, methotrexate and 5-fluorouracil (38).

Development of techniques to disrupt the expression of single genes in molecularly engineered cells has identified a number of previously unsuspected genes in various organisms in yeast, Dictyostelium, and mammalian cells, that control sensitivity to cisplatin. Genes that mediate sensitivity to cisplatin include the DNA mismatch repair, the sphingosine-1-phosphate

lyase 1, the Golgi vesicular membrane-golvesin, cAMP-specific phosphodiesterases, the regulatory subunit of the cAMP-dependent protein kinase (PKA), the Lyn tyrosine kinase, and a photolyase (reviewed in 39). DNA damage leads to simultaneous activation of proapoptotic and survival pathways in a time-dependent, hierarchical manner. Every transition in this response network results from a perturbation of the steady-state levels of intracellular second messengers such as Ca^{2+}, cAMP, cGMP, sphingosine 1-phosphate/ceramide and inositol polyphosphates (39).

Upregulation of activator protein-1 (AP-1) transcription factor has also been linked to chemotherapeutic resistance. AP-1 inhibition using an adenovirus expressing a dominant negative that inhibits AP-1 DNA binding was able to reverse drug resistance with significant decrease in cell viability in the KB85 multidrug-resistant and the A2780/CP70 cisplatin-resistant cells at drug doses normally not lethal to the cell. Thus, AP-1 is a therapeutic molecular target and inhibition of AP-1 DNA binding may be of clinical value in treating chemotherapeutic resistance (40). Up-regulation of Upstream Binding Factor (UBF), an RNA polymerase I-specific transcription factor, was detected in 12 of 17 clinical hepatocellular carcinoma samples comparing to the paired normal liver tissues. Contrary to AP-1, upregulation of UBF increased sensitivity to cisplatin in cell cultures. Downregulation of UBF with antisense oligodeoxynucleotides caused weak apoptosis without DNA laddering or cleavage of poly (ADP-ribose) polymerase and altered the expression of 30 genes (41). A 2.7-fold higher expression of the antiapoptotic protein $BclX_L$ in the human ovarian carcinoma cell line CH1, which are relatively sensitive to cisplatin, followed by subcutaneous inoculation of these cells into xenograft mouse models conferred significant resistance to both paclitaxel and cisplatin in comparison with parent, nontransfected tumors. Whereas the parent non-BclXL transfected tumors were highly responsive to either drug, and disappeared for at least 50 days post treatment, tumors overexpressing BclXL grew back after 30 and 20 days after treatment with paclitaxel and cisplatin, respectively. Such studies suggest examination of an additional transcription factor in human specimens to determine responsiveness to chemotherapy treatment (42).

The role of bcl-2 in the susceptibility of the MCF7 ADR human breast carcinoma line overexpressing the P-170 glycoprotein was evaluated using four multidrug resistance (MDR)-related drugs (doxorubicin, vincristine, vinblastine, actinomycin D) and three MDR-non-related drugs (cisplatin, bischloroethylnitrosourea or BCNU, 5-FU). Bcl-2-overexpressing clones show increased resistance to cisplatin and BCNU, while no difference to 5-FU were observed between the control cells and bcl-2 transfectants. Surprisingly, bcl-2-overexpressing clones displayed an increased sensitivity compared with the control cells to all four MDR-related drugs. The increased resistance of the bcl-2 transfectants to cisplatin was correlated to their ability to prevent apoptosis, while the enhanced sensitivity to doxorubicin was associated with an increased doxorubicin accumulation and a decreased doxorubicin efflux. Thus, bcl-2 may have distinct biological effects depending on the anticancer drug used (43).

Human tumor cell lines (predominantly ovarian) with acquired resistance to cisplatin, the orally bioavailable analogue JM216, and the structurally hindered analogue ZD-0473 (see below), have been established and characterized for underlying mechanisms of resistance. The most frequently observed changes in resistant cell lines included amplification of 4q and 6q, followed by amplification of 5q using comparative genomic hybridization. These are potential loci of genes associated with platinum analogue resistance. Amplification of 12q was observed in cell lines made resistant to JM216 or ZD-0473 in which increased DNA repair appeared to be the major mechanism of resistance for both agents (44). Mechanisms of resistance in the human ovarian carcinoma cell lines included reduced drug transport and DNA platination, increased glutathione (GSH) levels and loss of the MLH1 DNA mismatch repair gene. Some mechanisms of resistance common to those described for cisplatin (decreased drug uptake, increased glutathione) have been observed for ZD0473 in addition to increased BCL2 levels and loss of the DNA mismatch repair protein MLH1. Metallothionein is a thiol-containing protein also linked with tumor resistance to cisplatin. Overexpression of metallothionein in a cell line by stable gene transfection resulted in 7-fold protection from cisplatin but only 1.3-fold from ZD0473 (11).

Overexpression of the mdrl gene has been observed in ovarian cancers. Cisplatin, doxorubicin or paclitaxel were found to induce mdr1 transcription in ovarian cancer cell lines using quantitative real-time RT-PCR. Thus, mdr1 induction by antineoplastics is one of the reasons for failure of ovarian cancer therapy and cisplatin resistance (45).

Four cell line models of acquired resistance to the clinically-used platinum drug, oxaliplatin, have been established using human tumor cell lines in vitro; two colon (HCT116 and HT29) and two ovarian (A2780 and CH1). Resistance in the two colon cell lines was unique to oxaliplatin itself among the platinum drugs. Acquired oxaliplatin resistance was not due to either reduced drug membrane transport or increased levels of glutathione in any of the four resistant lines. Following exposure to oxaliplatin, a lower level of platinum-DNA adducts was present in acquired oxaliplatin-resistant HT29 cells but no change in the levels of platinum-DNA adducts relative to the parent lines in the other cell lines were found. In an A2780 subline that had a 5-fold resistance to cisplatin, a 1.7-fold resistance to oxaliplatin and no resistance to AMD0473 there was loss of hMLH1 DNA mismatch repair gene and a p53phe172 mutation (46).

7. Prognostic markers to cisplatin resistance and disease progression

Patients can be classified as either platinum-sensitive or platinum-resistant depending on whether they have relapsed or progressed within 26 weeks of completing first-line platinum-based chemotherapy (47).

Expression of the mitogen-activated protein kinase phosphatase-1 (MKP-1) was a prognostic marker for shorter progression-free survival of patients with invasive ovarian carcinomas. Patients with carcinomas positive for MKP-1 had a median progression-free survival of only 18.3 months compared to 40.6 months for patients with carcinomas negative for MKP-1. The MKP-1 mRNA levels were strongly inducible upon treatment of OVCAR-3 cells with cisplatin. Thus, MKP-1 expression is a clinically useful marker to estimate patient

prognosis as well as the response to cisplatin chemotherapy. MKP-1 is involved in inactivation of MAP-kinase pathways, regulation of stress-responses, suppression of apoptosis and is regulated by inflammatory mediators (48). Specimens of ovarian carcinomas from 25 surgically treated patients who received postoperatively chemotherapy containing platinum were analyzed by the terminal deoxynucleotidyl transferase-mediated dUTP-nick end labeling (TUNEL) technique for apoptotic cells; patients with high apoptotic index had significantly shorter survival times; thus, apoptotic index can be predictive of treatment outcome in ovarian cancer (49).

8. p53 mutations and resistance to platinum chemotherapy

p53 plays a central role in controlling cell cycle checkpoint regulation, DNA repair, transcription, and induces apoptosis. Lack of p53 function impairs these cellular processes, and this may be a basis of resistance to chemotherapeutic regimens. Conclusive evidence shows that the presence of a functional wild-type p53 gene renders ovarian cancer cells sensitive to cisplatin. p53 frequently mutates in ovarian cancers. Epithelial ovarian cancer patients undergoing platinum-base chemotherapy showed marked differences in p53 levels and mutations; 83% of nonresponders to chemotherapy had mutations in the p53 gene compared with 16% for responders. Apoptotic index was significantly greater in tumors with wild-type p53 gene than those without the gene. p53 gene transfer markedly enhanced the sensitivity to cisplatin and cisplatin-induced apoptosis, but did not affect the sensitivity to paclitaxel nor paclitaxel-induced apoptosis in ovarian cancer cells that lacked the p53 gene (50).

The tumor suppressive activity of p53 seems to involve at least eight independent pathways: (i) Induction of the p21 gene which causes growth arrest both via inhibition of cyclin-dependent kinases and via inactivation of PCNA; PCNA is the accessory molecule to DNA polymerases and and its absence causes arrest of DNA synthesis at the replication fork. (ii) Induction of the death-promoting bax gene as a mechanism which eliminates oncogenic virus-infected and transformed cells. (iii) By a direct interaction of p53 with origins or replication preventing firing and initiation of DNA replication. (iv) Via binding of p53 to a number of important molecules involved in transcription (TATA box-binding protein or TBP, TFIIH). (v) p53 functions in DNA repair by patrolling the genome for small insertion deletion mismatches or free ends of DNA. (vi) p53 is able to attract RPA, an accessory to DNA polymerases and as well as TFIIH and RAD51 at the damaged DNA sites; TFIIH, RAD51, and RPA have a demonstrated role in DNA repair. (vii) p53 induces Gadd45 involved in the arrest of the cell cycle and Mdm2 which, after exceeding a threshold value in the cell associates with p53 to restrict its regulatory functions; thus, Mdm2 acts as a feedback loop for p53 to moderate its apoptotic and cell cycle restrictive functions. (viii) p53 specifically interacts with several viral proteins such as T antigen of SV40 and E6 of human papillomavirus (reviewed in 51). This interaction is thought to inhibit p53 functions during viral infection.

p53 is frequently mutated in late-stage ovarian cancer and loss of wild-type p53 has been proposed as a determinant of cisplatin chemosensitivity; studies in cell lines stably transfected with HPV-16 E6 (E6 protein is able to interact with wtp53 thus sequestering p53 and making it unavailable for other functions) have shown that the loss of functional p53 can increase cisplatin cytotoxicity. The transfected cell line was also 2- to 3-fold more sensitive to transplatin and ZD0473 whereas it was approximately equitoxic to JM335 compared to the parental cell line. Because platinum uptake was both cell lines after cisplatin treatment the differences in platinum sensitivity were arising from the rate of removal of platinum-DNA adducts which was reduced in E6 compared with the parental cell line after cisplatin damage (52).

Several genes have been identified that when disabled seem to either impair or enhance recognition of cisplatin adducts. The best known of these are the DNA mismatch repair genes, and hMSH2 in combination with one of its heterodimer partners binds specifically to cisplatin adducts (39). Lack of the Pms2 gene was associated with an increased sensitivity, ranging from 2-6-fold, to some types of anticancer agents including the topoisomerase II poisons doxorubicin, etoposide and mitoxantrone, the platinum compounds cisplatin and oxaliplatin, the taxanes docetaxel and paclitaxel, and the antimetabolite gemcitabine (53).

9. Cisplatin and carboplatin biodistribution and cellular localization

Microdialysis sampling of blood and extracellular fluid simultaneously in blood, kidney, liver, and tumor tissue in anesthetized rat models was used to determine the pharmacokinetics of cisplatin and carboplatin. After i.v. bolus drug administration, samples were collected every 10 min for 4-6 h using microdialysis probes implanted into the jugular vein, kidney, and either liver or subcutaneously growing breast tumors. Following a 10 mg/kg dose of cisplatin, peak renal concentrations (80 µg/mL) always exceeded peak plasma (13 µg/mL) and hepatic (10 µg/mL) concentrations. For carboplatin, doses of 30 mg/kg also resulted in high peak renal concentrations (89 µg/mL) but peak hepatic carboplatin concentrations were increased significantly, resulting in a disproportionate 3.5-fold increase in mean AUC at 30 mg/kg compared to the 20 mg/kg dose suggesting a possible mechanism for high-dose carboplatin-induced hepatic toxicity. Tumor cisplatin and carboplatin AUCs were similar to that in the circulation, but variable, ranging from 52 to 109% of the corresponding plasma AUCs (54). The pharmacokinetics of total and unbound plasma carboplatin from 75 children (1-17 years old, 10 children with unilateral nephrectomy) treated using 1-hour daily infusions for various malignancies; the average population values for total unbound carboplatin clearance, CL(U), and distribution volume of unbound carboplatin, VI, were 3.87 l/h and 6.26 l/h, respectively (55).

Cellular localization of cisplatin in an ovarian carcinoma cell line was determined taking advantage of the electron-dense nature of platinum and electron microscopy. Platinum spots were detected in contact with the plasma membrane and the nuclear envelope as well as in the cytoplasm and nuclear matrices. No sequestration in intracellular vesicles was observed, thereby supporting that phagocytosis and receptor-mediated endocytosis were not occurring. Cisplatin rapidly accumulated in the cell consistent with passive diffusion rather than endocytosis membrane translocation (56).
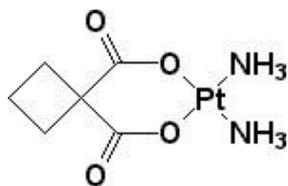
Figure 3. Structure of carboplatin

Recent clinical trials using cisplatin and carboplatin are being reviewed in the following article (33).

10. Carboplatin (Paraplatin)

Following the introduction of cisplatin and the demonstration of its importance in the treatment of testicular and ovarian cancer, there was a need to develop less toxic analogues. Early studies led to the clinical development of the less toxic analogue carboplatin (Fig. 3), oxaliplatin and JM216, the first orally administerable platinum drug. In recent years, the focus has been on two lead complexes designed to overcome the major mechanisms of tumour resistance to cisplatin: JM335 and ZD0473.

Carboplatin (Paraplatin) proved markedly less toxic to the kidneys and nervous system than cisplatin and caused less nausea and vomiting, while generally (and certainly for ovarian cancer) retaining equivalent antitumor activity. However, hematological adverse effects are more frequent with carboplatin than with cisplatin. The dosage of carboplatin can be determined according to the theory of Calvert *et al* (57) and is expressed as area-under-the-concentration-time-curve (AUC) in units of mg/mL.min. In many situations, carboplatin is now the drug of choice in view of the improved quality of life it offers to the patients compared to cisplatin but the debate "carboplatin versus cisplatin" continues and the decision of the medical oncologist may be affected by patient cases such as kidney function.

Carboplatin constitutes a reasonable alternative to cisplatin in a combination with gemcitabine, since it shows synergy with gemcitabine *in vitro*, is easier to use in ambulatory patients, and has a better nonhematologic toxicity profile. The combination of gemcitabine (Gemzar) and carboplatin, initially hampered by unacceptable platelet toxicity, has gained increasing acceptance against NSCLC. The combination of docetaxel with irinotecan and carboplatin has achieved a 1-year survival rate of 55%. The response rate was approximately 20% with the single-agent pemetrexed and approximately 40% in combination with cisplatin against NSCLC (reviewed in 33). Paclitaxel plus carboplatin was equally active and well tolerated compared to gemcitabine plus paclitaxel in a phase III randomized trial and gave response rates of 28.0% versus 35.0% (58). A Phase III randomized trial in 618 patients with advanced NSCLC gave a response rate of 25% (70 of 279) in the paclitaxel/carboplatin arm and 28% (80 of 284) in the paclitaxel/cisplatin arm (59). The use of vinorelbine, gemcitabine, paclitaxel and docetaxel in combination with cisplatin or carboplatin against NSCLC has increased by as much as 10% the overall survival at one year. Carboplatin / paclitaxel-based combination chemotherapy has become a very popular combination in the US against advanced

NSCLC and has advantages to the older cisplatin-based chemotherapy (reviewed in ref. 33).

A phase I study against advanced carcinoma has been completed using a weekly schedule of carboplatin at a fixed dose of AUC = 2 mg/mL/min and paclitaxel at escalating dose levels starting at 135 mg/m^2 per week for 6 weeks followed by a 2-week break per cycle. MTD was defined as the highest dose level at which less than 50% of patients developed unacceptable toxicity. Dose escalation was halted due to neutropenia and/or Grade 2/3 neuropathy. The MTD was 135 mg/m^2 paclitaxel / carboplatin AUC 2 for patients with previous chemotherapy exposure and 150 paclitaxel / carboplatin AUC 2 for chemotherapy-naive patients (60).

Carboplatin is a safe and effective first-line treatment for women with advanced ovarian cancer as deduced from four large randomized trials of paclitaxel in combination with platinum against a platinum-based control treatment representing 3588 patients (reviewed in ref. 61). Carboplatin as single agent has demonstrated a 17% response rate against measurable hormone refractory prostate cancer. Hormone refractory prostate cancer has been treated with combination chemotherapy using paclitaxel, estramustine phosphate and carboplatin to an area under the curve of 6 on day 1 of a 4-week cycle (62). Children with Wilm's tumor (nephroblastoma) were treated with high-dose melphalan, etoposide and carboplatin and autologous peripheral blood stem cell rescue in order to improve their probability of survival (63). Anaplastic astrocytomas and glioblastomas were treated with intravenous administration of carboplatin (300 mg/m^2) on day 1 and etoposide (60 mg/m^2) on day 1 to 5, repeated every 6 weeks (64). A combination of paclitaxel, etoposide and carboplatin is an often used regimen against small-cell lung cancer (65). Docetaxel combined with cisplatin gives an overall response rate of 33%-46% compared to 30%-48% of docetaxel combined with carboplatin (reviewed in 66).

In a phase II study carboplatin had a more favorable therapeutic index than cisplatin, particularly with regard to nonhematologic toxicities, and had proven activity in combination with docetaxel against NSCLC (67). Unlike taxane based regimens, there is no need for steroid premedication, and neurotoxicity and alopecia are absent in Gemzar plus carboplatin regimens. A randomized trial of cisplatin plus paclitaxel versus carboplatin plus paclitaxel did not detect a significant difference in survival between these regimens (reviewed in 68). Combinations of gemcitabine/paclitaxel/cisplatin against advanced urothelial cancer have high levels of activity with overall and complete response rates of 76% and 26%, respectively whereas combinations of gemcitabine/paclitaxel/carboplatin have overall and complete response rates of 68% and 32%, respectively (69). A Japanese study on 110 patients with epithelial ovarian cancer using carboplatin at a dose of AUC 5 and a dose escalation of paclitaxel at levels of 150, 175 and 200 mg/m^2 observed grade 4 neutropenia as the DLT and a response rate of 66.7% (70).

11. Oxaliplatin (Eloxatine)

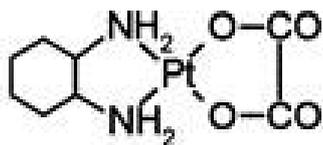Oxaliplatin, (1R,2R-Diaminocyclohexane)oxalatoplatinum(II) (Fig. 4),

Figure 4. Structure of oxaliplatin

produces the same type of inter- and 1,2-GG intrastrand cross-links as cisplatin but has a spectrum of activity and mechanisms of action and resistance different from those of cisplatin and carboplatin. Oxaliplatin has a non-hydrolyzable diaminocyclohexane (DACH) carrier ligand which is maintained in the final cytotoxic metabolites of the drug. The cellular and molecular aspects of the mechanism of action of oxaliplatin have not yet been fully elucidated. However, the intrinsic chemical and steric characteristics of the DACH-platinum adducts appear to contribute to the lack of cross-resistance with cisplatin (reviewed in ref. 71).

Alkaline hydrolysis of oxaliplatin gives the oxalato monodentate complex (pK_a 7.23) and the dihydrated oxaliplatin complex in two consecutive steps. The monodentate intermediate is assumed to rapidly react with endogenous compounds (72). The crystal structures of oxaliplatin bound to a DNA dodecamer duplex with the sequence 5'-d(CCTCTGGTCTCC) has been reported (73). The platinum atom forms a 1,2-intrastrand cross-link between two adjacent guanosine residues (shown in boldface) bending the double helix by approximately 30 degrees toward the major groove. The crystallography provided structural evidence for the importance of chirality in mediating the interaction between oxaliplatin and duplex DNA (73). Oxaliplatin, like cisplatin, adduct lesions are repaired by the nucleotide excision repair system. Oxaliplatin, like cisplatin, is detoxified by glutathione (GSH)-related enzymes. ERCC1 and XPA expression was predictive of oxaliplatin sensitivity in six colon cell lines *in vitro* (74).

Oxaliplatin has shown a wide antitumor effect both *in vitro* and *in vivo*, a better safety profile than cisplatin and a lack of cross-resistance with cisplatin and carboplatin. The anticancer effects of oxaliplatin are optimized when it is administered in combination with other anticancer agents, such as 5-fluorouracil, gemcitabine, cisplatin, carboplatin, topoisomerase I inhibitors, and taxanes (reviewed in 75). Oxaliplatin has a unique pattern of side effects and besides neurotoxicity they include hematologic toxicity and gastrointestinal tract toxicity. Grade 3/4 neutropenia occurred in 41.7% of patients in a phase III clinical trial. Nausea and vomiting is usually mild to moderate and readily controlled with standard antiemetics. Nephrotoxicity is mild allowing administration of oxaliplatin without hydration (76). Sporadically, severe side effects may be observed such as tubular necrosis (77).

Oxaliplatin in combination with 5-fluorouracil, has been recently approved in Europe, Asia, and Latin America for the treatment of metastatic colorectal cancer.

The pharmacokinetics of the free fraction of oxaliplatin in blood were evaluated in 10 patients given 85 mg/m^2 of oxaliplatin using an infusion time of 2 h. The maximal blood concentration (C_{max}) was 1.44 µg/mL. Oxaliplatin's half-life in
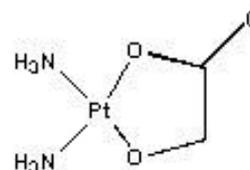

Figure 5. Structure of nedaplatin

this study, determined by liquid chromatography in combination with postcolumn derivatization, was 14 min which is in a sharp contrast to previously reported elimination half lives obtained by analysis of the platinum content in plasma and ultrafiltrate. The AUC was 161 µg min/mL, the clearance (CL) was 32.1 L/h/m^2 and the distribution volume (V_{ss}) was 0.26 L/kg (78). Oxaliplatin improved the response rate and progression-free survival when given with 5-fluorouracil/folic acid for the treatment of advanced colorectal cancer, whereas its activity in other tumor types is under investigation. The dose-limiting side effect of oxaliplatin is neurotoxicity; sodium channel inactivation kinetics are altered after exposure of animals to oxaliplatin. Results from preliminary clinical studies indicate that the sodium channel blockers carbamazepine and gabapentin may be effective in preventing neurotoxicity (79).

A phase II study using combination of raltitrexed (Tomudex; AstraZeneca) and oxaliplatin 130 mg/m^2 every 3 weeks in patients with diffuse malignant pleural mesothelioma as first line treatment gave a 20% partial response and 46% stable disease in the patient population with an acceptable tolerability profile (80). Oxaliplatin, 5-FU and leucovorin have a synergistic activity on metastatic colorectal cancer. Forty-six patients previously treated with chemotherapy gave 1 complete response (2.2%) and 14 partial responses (30.4%) after this regimen using 50 mg/m^2 oxaliplatin and with all drugs administered on days 1 and 2, every 14 days (81). The efficacy and tolerance of the bimonthly administration of oxaliplatin at 100 mg/m^2 on day 1 in combination with high-dose leucovorin (500 mg/m^2 on days 1 and 2) and 5-FU (1,750 mg/m^2/d as a 22-hour continuous intravenous infusion on days 1 and 2) in patients with advanced colorectal cancer who did not respond or whose disease progressed within 3 months after front-line treatment was studied. Complete response was achieved in one out of 41 (2.4%) and partial response in 6 out of 41 patients (14.6%) whereas stable disease was observed in 15 of 41 (36.6%) patients; the median time to tumor progression was 8.5 months, the median overall survival was 12 months and the probability for 1-year survival was 42.9% (82).

12. Nedaplatin

Nedaplatin or cis-diamine(glycolato)platinum(II) (Fig. 5) is manufactured by Shionogi & Co. Ltd, Osaka, Japan.

13. Preclinical studies with nedaplatin

A combination therapy of nedaplatin (NDP) with paclitaxel (TXL) against Lewis lung carcinoma was intriguing. When

nedaplatin was given prior to TXL (NT therapy) it resulted in severe loss of body weight followed by frequent toxic deaths in mice. On the contrary, administration of TXL prior to NDP (TN therapy) resulted in synergistically enhanced inhibition of tumor growth with less toxicity compared with the NT therapy. The combination effect of NDP plus TXL was significantly higher than that of cisplatin plus TXL or carboplatin plus TXL in mice (84).

A human lung cancer subline was established that was 7 times more resistant to gemcitabine than parent cells and cells were implanted to athymic mice. Treatment of mice with a combination of nedaplatin and gemcitabine showed synergistically enhanced inhibition of tumor growth that was superior to the best effect of either monotherapy. These studies suggest the effectiveness of the combination of nedaplatin with gemcitabine against gemcitabine-refractory human lung cancers (85). The antitumor activity of a combination of paclitaxel followed by nedaplatin against SK-OV-3 human ovarian cancer cells in animal models was evaluated. Paclitaxel was injected i.v. daily for four days and either nedaplatin, carboplatin or cisplatin were injected i.v. once after the paclitaxel treatment, into tumor-bearing mice. The combination of paclitaxel plus nedaplatin was synergistic and superior to that of other combinations (86). The effect of cell-cycle nonspecific anticancer agents such as nedaplatin is believed to depend on the area under the drug concentration-time curve (AUC) (87).

14. Clinical studies with nedaplatin

When nedaplatin was used as a single agent in a phase I study, the dose-limiting toxicity (DLT) was thrombocytopenia and the recommended dose was 100 mg/m^2. The pharmacokinetics and pharmacodynamics of serum platinum after nedaplatin showed that peak serum platinum concentrations were dependent on infusion times. The concentration of serum platinum after nedaplatin tended to stay at a high level for a long time in patients with renal dysfunction or ascites fluid (88). Nedaplatin appears to have less renal toxicity and higher efficacy for uterine cervical cancer than cisplatin in human trials. A Phase I study was set to determine the dose-limiting toxicity (DLT), maximum tolerated dose (MTD) and recommended dose (RD) of nedaplatin in combination with 5-fluorouracil. 5FU was administered to 38 patients at a fixed dose (700 mg/m^2/d on days 1-5) and NDP was administered on day 6 at an initial dose of 80 mg/m^2, which was subsequently increased to 100, 120, 130, 140, 150, and 160 mg/m^2. The DLT of NDP was leukopenia and its MTD and RD were 160 and 150 mg/m^2, respectively. There were 19 responders (50%, 19/38) achieving partial response or complete response (89).

Phase I studies using a combination of gemcitabine and nedaplatin on 21 chemotherapy-naive patients with advanced NSCLC (stage IV) and a performance status of 0-2 showed grade 3 or 4 thrombocytopenia (19% of patients), and grade 3 or 4 neutropenia (24% of patients). Nonhematologic toxicities were mild. Grade 3 hepatic dysfunction occurred in 3 patients. The doses of gemcitabine (days 1, 8) and nedaplatin (day 1) studied were 800/60, 800/70, 800/80, 1000/80, and 1000/100 mg/m^2, repeated every 3 weeks. Although the MTD was not reached even at the highest doses, the recommended doses were 1000
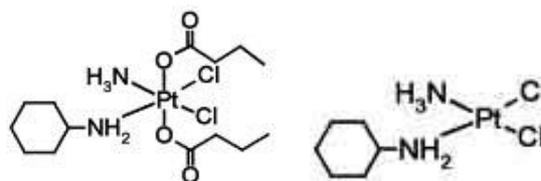

Figure 6. Structure of JM216 (left) and JM118 (right) (94)

mg/m^2 of gemcitabine and 100 mg/m^2 of nedaplatin for phase II studies (90). A combination chemotherapy of intravenous nedaplatin and intraarticular cisplatin with transcatheter arterial embolization on 32 patients with cervical cancer was studied. Previous trials have shown that intraarterial cisplatin is more effective than intravenous cisplatin in the treatment of cervical cancer. Nedaplatin (30-70 mg/m^2) was administered intravenously on day 1 and cisplatin (70 mg/m^2) was administered intraarticularly via both uterine arteries on day 3 using the Seldinger method. Transcatheter arterial embolization was then performed. This course of treatment was repeated every 3 weeks for 2-3 cycles. Partial response was found in 59% of patients (19/32) and complete response in 34% (11/32), with an overall response rate of 94% (30/32) defined by magnetic resonance imaging; 84% of patients showed an overall survival of 1 year and 77% an overall survival of 2 years (91). Unbound platinum concentrations in plasma after intravenous infusion of nedaplatin were measured for 187 courses in 145 patients with lung, esophageal, cervical and ovarian cancer undergoing clinical treatment. The formula obtained for predicting the platinum clearance (CL) using the creatinine clearance (CLcr) was CL=0.0836xCLcr+3.45, useful for estimating the CL for the first or second treatment with nedaplatin (92).

A phase II study of nedaplatin (80 mg/m^2, day 1) and vindesine (3 mg/m^2, days 1 and 8) every 3 to 4 weeks on 48 patients with relapsed or refractory NSCLC who had previously received chemotherapy, thoracic radiotherapy, and/or surgery showed the following results: a partial response of 3 out of 40 patients (7.5%) was observed among those who had received prior chemotherapy, compared to 4 out of 8 (50%) chemotherapy-naive patients. These data demonstrate the much higher effectiveness of chemotherapy regimens given as first line treatments and the gun smoke in low response rates in second and third line treatment points to chemoresistance developed during the first line chemotherapy (93).

15. JM216 (Satraplatin) and its metabolite JM118

JM216, or bis-aceto-ammine-dichloro-cyclohexylamine-platinum(IV) (Fig. 6) is the first orally administered platinum drug that is currently undergoing phase III clinical trials. It is rapidly metabolized to JM118 or *cis*-amminedichloro(cyclohexylamine)platinum(II) (Fig. 5) in the blood (97).

DNA damage inflicted by JM216 is being repaired *in vitro* with similar kinetics to those of cisplatin and oxaliplatin by the mammalian nucleotide excision repair pathway (94). A phase II trial of JM216 in patients with small-cell lung cancer (SCLC) using 120 mg/m^2/d for 5 consecutive days every 3 weeks gave grade 3 and 4 neutropenia in 15.9% and 3.7% of cycles,
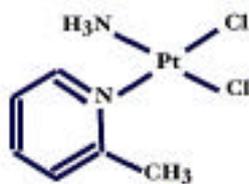
Figure 7. Structure of ZD0473

lymphocytopenia in 47.6% and 17.1%, and thrombocytopenia in 19.5% and 10.3% of cycles, respectively, whereas nausea, vomiting, and diarrhea were the most common nonhematologic toxicities. The tumor response rate was 10 of 26 (38%) with no complete responses and a median overall survival time of 210 days (95). Cell culture studies have shown that JM216 as well as its metabolite JM118 can partially circumvent intrinsic and acquired resistance to cisplatin suggesting a Pt-resistance mechanism based on tolerance or increased repair, rather than decreased initial Pt-DNA adduct formation (96). Satraplatin undergoes extensive biotransformation *in vivo* and very little intact parent drug remains in the systemic circulation following oral administration. *In vitro* studies where satraplatin was incubated with fresh human whole blood have shown a half-life for the disappearance of the drug of only 6.3 min and its conversion mainly to JM118 and platinated serum albumin whereas 62% of the added platinum was associated with red blood cells (97).

JM118 is the active form of JM216 and has been shown to form intrastrand cross-links and to have a greater cytotoxicity than cisplatin with respect to human ovarian carcinoma cells. JM118, binds to DNA similarly to cisplatin, forming intra- and interstrand cross-links between adjacent purine bases such as two isomeric 1,2-d(GpG) intrastrand cross-links. The X-ray crystal structure of the major adduct between this molecule and a DNA dodecamer at 2.4 Å resolution showed details of the distortion of the DNA duplex including a global bend angle of about 38° and a dihedral angle between platinated guanine bases of approximately 31° in a very similar manner to that of cisplatin and oxaliplatin. Therefore, differences in activity between these drugs are unlikely to result from gross conformational distortions in DNA structure following platinum intrastrand cross-link formation (98).

16. ZD0473 (formerly JM473 and AMD0473)

ZD0473, or cis-amminedichloro(2-methylpyridine) platinum (II) (Fig. 7), is a sterically hindered platinum (II) complex currently undergoing worldwide Phase II clinical studies. Myelosuppression is the dose-limiting toxicity at a dose of 130 mg/m^2 given i.v. every 3 weeks. ZD0473 relies on steric hindrance to overcome thiol-mediated detoxification. ZD0473 has been designed and synthesized by Johnson Matthey Technology and the Cancer Research Campaign (CRC) in UK and is under development as a potential treatment for cisplatin-resistant cancer. In June 2000, Deutsche Bank predicted sales of US $15 million in 2003 and in March 1999, Lehman Brothers predicted a 15% probability that the drug would reach worldwide markets, and be launched onto the market in 2003 (99).

In preclinical studies, ZD0473 showed evidence of an extended spectrum of anti-tumor activity overcoming platinum resistance mechanisms. ZD0473 exhibited superior circumvention of acquired oxaliplatin resistance in comparison to either cisplatin or the trinuclear platinum BBR3464 (46). ZD0473 shows promise against advanced epithelial ovarian cancer (reviewed in 100). The bulky methylpyridine ligand at its platinum center was thought to be responsible for its ability to overcome platinum resistance (101). The dichloro form of the drug exists in equilibrium with at least two aquated forms in plasma. A method that quantitatively converts the aquated species back to the dichloro form of the parent drug in human plasma ultrafiltrate samples and an isotope dilution LC/MS assay has allowed to detect ZD0473 at levels as low as 10 ng/mL. This was sensitive enough to detect drug levels in sera in a phase II study in which ZD0473 was administered to patients as an intravenous infusion at a dose of 150 mg/m^2 (102). ZD0473 may be usefully combined with various cytotoxics in the clinic, including paclitaxel, topotecan and gemcitabine as deduced from their synergistic effects on human ovarian carcinoma cell lines (103).

A phase II trial was undertaken to assess the antitumor activity of ZD0473 in ovarian cancer patients who had failed initial platinum-based therapy using 120 mg/m^2 ZD0473 as a 1-h intravenous infusion every 21 days. Patients were classified as either platinum-sensitive (n=35) or platinum-resistant (n=59) depending on whether they had relapsed or progressed within 26 weeks of completing first-line platinum-based chemotherapy. ZD0473 had side effects and 9% of patients withdrew because of treatment-related adverse events. Objective response rates for platinum-resistant and sensitive patients were 8.3 and 32.4%, respectively, and clinical benefit was observed in 76.5% of the sensitive patients (47).

17. BBR3464 or trinuclear platinum complex

The bifunctional DNA binding of the trinuclear platinum complex BBR3464 (Fig. 8) is characterized by the rapid formation of long range intra- and interstrand cross-links. BBR3464 forms 1,4-interstrand cross-links with the model double-stranded palindromic DNA octamer 5'-d(ATG*TACAT)$_2$-3', with the two platinum atoms coordinated in the major groove at the N7 positions of guanines that are four base pairs apart on opposite DNA strands. The significant characteristic of the structure is the lack of severe DNA distortion such as a kink, bend or unwinding of the helices suggesting non-recognition of its adducts by HMG-domain proteins; this finding implies significantly different biological consequences from those of cisplatin, carboplatin, oxaliplatin, nedaplatin and other mononuclear platinum complexes (104). Moreover, 1,4-interstrand crosslinks are not removed from DNA by nucleotide excision repair and could persist considerably longer in cells (105). On the contrary, intrastrand cross-links of BBR3464 create a local conformational distortion, are not recognized by high mobility group 1 proteins, but seem to be effective removed by nucleotide excision repair. Thus, the processing of the intrastrand cross-links of BBR3464 in tumor cells sensitive to this drug may not be relevant to its antitumor effects (106). Interaction and binding of BBR3464 (also of dinuclear platinum compounds with polyamine linkers) to DNA corresponds to their relative charge (2+ to 4+) and causes
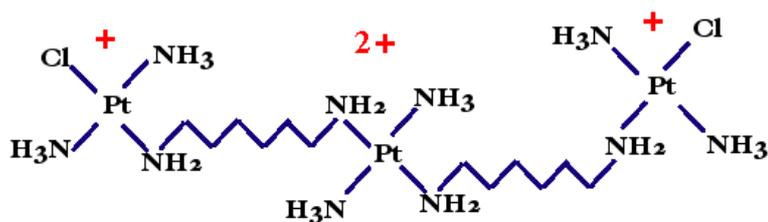
Figure 8. Structure of BBR3464.

unwinding of supercoiled DNA circles (107). Second-generation analogues of BBR3464 based on polyamine-bridged dinuclear platinum compounds are under preclinical evaluation whereas their blocked-polyamine compounds are 2-3 orders of magnitude less cytotoxic than the parent drug based on the pro-drug concept for greater selectivity and/or oral delivery (108). The reaction of BBR3464 with single-stranded DNA and RNA was faster than that with duplex DNA and produced more drug-DNA and drug-RNA adducts (109); it is suggested here that BBR3464 has a preference for chromatin domains under torsional strain, for the actual replication fork and for transcribing genes.

Whereas the MTD was 4 mg/kg for cisplatin, the MTD for BBR3464 was 0.35 mg/kg in mouse xenografts. BBR3464 has been shown to circumvent the resistance to cisplatin in a panel of tumor cell lines and xenografts with acquired or intrinsic resistance to cisplatin. BBR3464 accumulation and DNA-bound platinum were higher than those observed for cisplatin in cell cultures. A number of elegant publications have studied the interaction of BBR3464 with DNA *in vitro* (110,111), with cell cultures (112) and its antitumor efficacy in mouse xenografts (113).

Cell cycle analysis demonstrated a longer-lasting block in G2/M phase induced by BBR 3464 without the early S phase accumulation induced by cisplatin. The higher potency of BBR3464 correlated with an increased cellular platinum accumulation and DNA-adduct formation following BBR3464 exposure compared with cisplatin exposure. Studies on DNA mismatch repair deficient and proficient colon carcinoma cells were consistent with a lack of influence of the DNA mismatch repair status on BBR3464 cytotoxicity (114).

BBR3464 induced apoptosis to a lesser extent than cisplatin. Whereas cisplatin treatment resulted in the upregulation of p53, p21 and bax, only p21 induction was observed after BBR3464 treatment; thus, the two drugs seem to act through different mechanisms (115-117). The chemical feature of a diamine linker containing an internal charge contributes significantly to the anticancer profiles of the trinuclear platinum complex, BBR3464 (118). Loss of mismatch repair proteins (hMLH1, hPMS2) or overexpression of ERCC1 was not detrimental for the cellular sensitivity to BBR3464 in two human ovarian cancer cell lines sensitive or resistant to cisplatin (119).

BBR3464, evaluated in phase II trials, has 100 times the cytotoxicity of cisplatin. In a Phase I clinical trial 14 patients with advanced solid tumors not responsive to previous antitumor treatments received BBR3464 on a daily schedule for 5 consecutive days repeated every 28 days. BBR3464 was given as a one-hour infusion with pre-and post-treatment hydration at a starting dose of 0.03 mg/m^2/day. Dose was escalated four times up to 0.17 mg/m^2/day. Short-lasting neutropenia and diarrhea of late onset were dose-limiting and defined the MTD at 0.12 mg/m^2. The higher than expected incidence of neutropenia and GI toxicity observed with BBR3464 might be related to the prolonged half-life and accumulation. Neither neuro- nor renal toxic effects were observed and nausea and vomiting were rare. Approximately 10% of the equivalent dose of BBR3464 (2.2%-13.4%) was recovered in a 24-hour urine collection (120).

18. SPI-77

The STEALH liposome-encapsulated cisplatin SPI-77 is a liposomal formulation of cisplatin developed by ALZA (121,122). SPI-77 displays a different pharmacokinetic behavior compared to cisplatin with a half-life of 134 h and urine excretion reaching only 4% of the total dose in 72 h in Phase I clinical trials (123). The toxicity profile of SPI-77 is also mild compared to cisplatin (123,124). SPI-77 administered at doses of 260 mg/m^2 did not show benefit against non-small cell lung cancer (125) or head and neck cancers (126).

SPI-77 has also been compared to the standard carboplatin therapy in dogs with osteosarcoma. The regimens were 350 mg/m^2 for SPI-77 and 300 mg/m^2 for carboplatin both administered i.v. every 3 weeks for four treatments. The safe administration of five times the maximally tolerated dose of free cisplatin to dogs in its SPI-77 formulation and without concurrent hydration gave some benefits over carboplatin but did not translate into significantly prolonged median disease-free (MDF) or overall survival (OS) (127). The lipid to drug ratio in SPI-77 is 71.43 mg lipid per mg cisplatin (128). It appears at present that SPI-77 is failing to move successfully into a new pharmaceutical (125).

19. Lipoplatin

Lipoplatin is a liposomal cisplatin formulation by Regulon currently undergoing Phase II evaluation with over 100 patients treated. A Phase I clinical trial on 19 patients with advanced cancers using escalating doses of Lipoplatin as second or third line treatment has been completed. Lipoplatin showed an increased time of circulation in sera of patients with a half-life of 36h following an 8-h i.v. infusion. For comparison, cisplatin is excreted in the urine with a half-life in blood circulation of about 6 h following bolus or i.v. infusion. The side effects of Lipoplatin

using 100 mg/m^2 every 14 days included mild myelotoxicity / hematological toxicity (Grade I-III in 30% of the patients), mild GI tract toxicity, and mild nausea/vomiting (Grade I-III in 20% of the patients) as well as an abdominal and back pain in 15% of treatments observed during the first 15 min of infusion that usually lasted for 10 min and went away spontaneously without pain killers. There was no ototoxicity, renal toxicity or other side effects observed at this dosing schedule. In addition, there was no need for pre and post hydration during treatment with Lipoplatin as opposed to cisplatin treatments (Boulikas and Stathopoulos, unpublished data).

It is interesting to note that three patients in this Phase I study with mild renal insufficiency and with serum creatinine of 1.5-2.2, who were treated with a dose of 100 mg/m^2, showed no increase in serum creatinine and no aggravation of hematological toxicity. Two of 19 (10.5%) patients (one with gastric and the other with pancreatic cancer, both with lung metastasis) achieved a partial response and 10 of 19 (53%) achieved stable disease and clinical benefit during a follow up of 2-5 months in the phase I clinical trial (Boulikas and Stathopoulos, unpublished data).

The advantage of Lipoplatin appears to arise from its 2- to 50-fold higher concentration in human tumors compared to normal human tissues in biopsies, measured as total platinum with atomic absorption (Stathopoulos and Boulikas, unpublished data). Thus, Lipoplatin, like the Doxil/Caelyx liposomal formulation of doxorubicin by Alza, is endowed with tumor targeting properties; the Lipoplatin formulation can attain a higher concentration in tumors via its preferential extravasation through the altered and compromised tumor vasculature. In order to achieve this property liposomes that enclose chemotherapy drugs must have a diameter below 130 nm, long circulation properties and the ability to escape immune surveillance.

The dose limiting toxicities, still under evaluation, are most likely to be neutropenia. Also under clinical evaluation is a dose intense Lipoplatin monotherapy using 100 mg/m^2 every week for 5-10 cycles.

Several Phase I/II clinical trials under way. One Phase II clinical trial is using Lipoplatin monotherapy against NSCLC as second line treatment. A different Phase II compares Lipoplatin with radiation therapy against NSCLC using a weekly dose intense Lipoplatin monotherapy. Four other ongoing Phase I/II clinical trials explore the MTD, DLT, RD, time to disease progression and response rates of Lipoplatin in its combination with the standard dose and treatment schedule of paclitaxel or gemcitabine against NSCLC, urinary bladder, head & neck and pancreatic cancers.

20. Experimental liposome formulations of platinum compounds

The antitumor activity of cisplatin, encapsulated into transferrin-conjugated polyethylene glycol liposomes (Tf-PEG liposomes) was studied in nude mice with peritoneal dissemination of human gastric cancer cells. Small unilamellar Tf-PEG, PEG or DSPC/CH liposomes (bare liposomes) encapsulating cisplatin were prepared by reverse-phase evaporation followed by extrusion. The Tf-PEG liposomes were internalized into tumor cells by receptor-mediated endocytosis as shown by electron microscopy. Uptake of Tf-PEG liposomes into the liver and spleen was significantly lower than that of bare liposomes and had antitumor properties in nude mice xenografts that were better than free cisplatin (129). Niosomes of cisplatin using span 60 and cholesterol have been used to investigate their antimetastatic activity in mouse B16F10 melanoma and showed significant reduction in the number of metastatic lung nodules (130).

Trans(+/-)-1,2-diaminocyclohexaneplatinum(II) complexes of malonate derivatives were formulated into liposomes. Due to the lipophilicity of the malonatoplatinum complexes, the entrapment efficiency of drugs within the liposomes was over 90% allowing preparation by lyophilization, and reconstitution in aqueous solution. The liposomal dibenzylmalonate derivative was much more cytotoxic to both cisplatin sensitive (A2780) and cisplatin resistant (A2780/PDD) human ovarian carcinoma cells than the corresponding diallylmalonate and allylbenzylmalonate complexes because of the hydrophobic benzyl substituent in the malonato leaving group (131).

21. Experimental cisplatin-lipid conjugates

A novel bile acid-cisplatin complex, Bamet-R2, or cis-diamminechlorocholylglycinateplatinum (II), with liver vectoriality, has been synthesized with the aim to overcome cisplatin resistance. This complex had increased water solubility by encapsulation into liposomes and enhanced uptake by liver tumor cells. Bamet-R2 was effectively incorporated into liposomes with an increase in the concentration of the drug by more than 6 million-fold compared with that in the initial free solution; this is one thousand-fold higher than the encapsulation obtained for cisplatin by the same laboratory (132). A role for organic anion transporting polypeptide (OATP), and organic cation transporter (OCT) in Bamet-R2 uptake, but not in cisplatin uptake, was supported in experiments using *Xenopus laevis* oocytes or Chinese hamster ovary cells and overexpression of OATP and OCT. Bamet-UD2, or cis-diammine-bisursodeoxycholate-platinum(II), exhibited a similar behavior. This mechanism may determine the ability of the cholylglycinate-cisplatin derivatives to accumulate in liver tumor cells and/or be taken up and efficiently excreted by hepatocytes (133).

A number of older formulations of platinum compounds have not resulted, or advanced, in clinical trials. A lipophilic cisplatin derivative, cis-bis-neodecanoato-trans-R,R-1,2-diaminocyclohexane platinum (II) (NDDP) formulated in conventional liposomes was shown to be nonnephrotoxic in humans, not cross-resistant with cisplatin in different *in vitro* and *in vivo* systems, and more active than cisplatin against murine models of experimental liver metastasis. (134). NDDP was also formulated in liposomes composed of phosphatidylcholine, cholesterol and monosialoganglioside or polyethyleneglycol conjugated to phosphatidylethanolamine with prolonged circulation times; there was a 3-fold increase in tumor accumulation of these liposomes as compared to the conventional phosphatidylcholine / cholesterol liposomes; however, these formulations were ineffective in inhibiting tumor growth (135). These formulations have been instructive to our comprehension of liposomal formulations of cisplatin-lipid conjugates and their behavior against animal tumors, but have not progressed to successful human clinical trials and pharmaceuticals.

22. Delivery of cisplatin with polymers in preclinical and clinical stage

Local and sustained release of cisplatin may have discrete advantages to obtain a high concentration of the drug near or inside a tumor. Cisplatin formulations into gel-type materials suitable for intratumoral injection have been tested in several laboratories. In general these methods suffer from inefficient loading of the drug and other hurdles relating to its release mode and overall toxicity of the formulation. Malignant bone tumors are treated with surgical therapy and simultaneous systemic chemotherapy. In order to overcome the toxicity of this approach the bone-cementing apatite (calcium phosphate) was used for a cisplatin formulation to develop an implant and maintain high concentrations of cisplatin at local sites in animals toward improving local structural weakness after tumor resection and treating residual malignant bone tumors. The in-vitro cumulative release ratio of an implant containing 20% cisplatin was over 60%, and a release rate of 0.1 mg/day was maintained. Rabbits implanted with a 10% cisplatin-apatite cement, showed high platinum levels in local bone marrow 6 weeks after implantation (3200 μg/tissue.g) whereas the levels in liver and kidneys were at the 2-3 μg/tissue.g range (136). Camptothecin was coupled to poly(L-glutamic acid) through the C20(S)-hydroxyl group. Although the camptothecin-polyG was less effective in inhibiting cell growth than free camptothecin in cell lines, it showed better antitumor activity and tolerability than camptothecin in a human lung cancer animal xenograft (137).

A new experimental approach at the preclinical stage has used injectable, biodegradable microspheres releasing carboplatin, doxorubicin, or 5-fluorouracil to suppress the growth of solid tumors. Chemotherapeutic microspheres were injected into the tumor center or multiple sites along the outer perimeter of the tumor in rats and produced a significant, dose-related suppression in tumor growth. Moreover, five temporally-spaced microsphere treatments along the tumor perimeter (with either doxorubicin or 5-fluorouracil microspheres) completely eradicated 100% of the subcutaneous tumors and 40-53% of the intramuscular tumors (138).

The adsorption of cisplatin by slurries of hydroxyapatite and its release for local treatment of malignant tumors were found to depend significantly on the ionic composition of the aqueous media used. In chloride-free phosphate buffered solutions or in a Tris buffered solution more cisplatin was adsorbed by the hydroxyapatite crystals; it was the hydrated derivatives of cisplatin which were involved in the adsorption of cisplatin by hydroxyapatite crystals. Approximately 33% of the total bound cisplatin was released after 4.25 days and these systems might find applications for the slow and local release of cisplatin *in vivo* (139).

PLGA-mPEG nanoparticles of cisplatin were prepared by a double emulsion method and characterized with regard to their morphology, size, zeta potential and drug loading. Although intravenous administration of these cisplatin nanoparticles in mice resulted in prolonged cisplatin circulation in blood, they suffered from loading efficiency for therapeutic applications (140). Degradable starch microspheres in an aqueous crystal suspension were used in clinical trials to achieve intensification of intraarterial chemotherapy of head and neck cancer with high-dose cisplatin. A new method of chemoembolization was established which could be routinely used without the drawbacks of earlier methods such as low drug dosage due to early occlusion of the small head and neck vessels and danger of local damage (141). The biodegradable 6-carboxylcellulose polymer (cisplatin-depot) has also been used in patients with glioblastoma multiforme (142).

23. Discovery of novel platinum compounds

A novel platinum (II) coordination complex has been synthesized containing diaminocyclohexane as carrier ligand and glycolic acid as a leaving group. This complex has demonstrated higher efficacy and lower cytotoxicity compared to cisplatin against human ovarian adenocarcinoma and prostate carcinoma cell lines, rabbit proximal renal tubular cells and human renal cortical tissues *in vitro* (83). The synthesis of new sterically hindered trans- and cis-diaminedichloroplatinum(II) complexes with monofunctional piperidine and piperazine as amine ligands has been described. The introduction of the positively charged piperazine ligand, which allows retaining of the classic platinum coordination sphere gives to the molecule increased solubility compared to cisplatin. Replacement of one NH_3 of the inactive transplatin by the aromatic planar ligand (4-picoline) or by the aliphatic nonplanar heterocyclic ligand (piperidine) or replacing both NH_3 groups with a 4-picoline ligand and a piperidine or piperazine ligand significantly increased the cytotoxic activity of these complexes (143). Overall, the charged complexes cis/trans-$[PtCl_2(piperazine)(Am)]$ (where Am = NH_3, n-butylamine, isopropylamine, 4-picoline, piperidine, or piperazine) circumvented cisplatin resistance in ovarian cancer cell lines, were water soluble, were taken up by cancer cells much more rapidly than cisplatin and were bound to cellular DNA and to calf thymus DNA much faster than cisplatin or transplatin (144). For robotic drug synthesis and discovery, platinum drug candidates were generated through the use of automated synthesis and reaction products were screened for activity in a high-throughput transcription assay. Using this powerful technology over 3,600 reaction products were screened for their ability to inhibit transcription of beta-lactamase in the BlaM HeLa cell line by monitoring cleavage of a lactam ring linking the two halves of a fluorescent resonance energy transfer dye. Of the four species identified, cis-$[(isopropylamine)_2PtCl_2]$, cis-$[(cyclobutylamine)_2PtCl_2]$, and cis-$[ammine(cyclobutylamine)PtCl_2]$, were previously determined to be active cisplatin analogs whereas the fourth compound, cis-$[ammine(2-amino-3-picoline)PtCl_2]$, represents a new kind of antitumor drug candidate similar to ZD0473 (145).

24. The promise of gene therapy

Gene therapy approaches have suffered from the inadequate transduction efficiencies of replication-defective vectors. Replication-competent vectors, particularly adenoviruses that cause cytolysis as part of their natural life cycle, represent an emerging technology that shows considerable promise as a novel treatment option, particularly for locally advanced or recurrent

cancer. Especially promising are adenoviruses that selectively replicate in tumor cells that have shown promising preliminary results in clinical trials, especially in combination with chemotherapy (146, reviewed in 51). Liposomal formulations of genes may overcome significant hurdles in gene therapy applications in a clinical setting (reviewed in 147). Many of those are in preclinical or cell culture studies and very few have surfaced to human clinical trials. Preliminary data from clinical studies using the liposomal encapsulation of replication incompetent Semliki Forest Viruses carrying therapeutic genes, such as the human interleukin-12 gene, showed promise in Phase I/II clinical trials as a cancer immunotherapy regimen (148).

Endovascular microcoils, used in interventional procedures to treat cerebral aneurysms, have been successfully used as an experimental gene delivery system. Anti-adenoviral monoclonal antibodies were covalently attached to the collagen-coated surface of either platinum or polyglycolic acid microcoils and used to tether replication-deficient adenoviruses encoding green fluorescent protein or beta-galactosidase on animal models (149).

An emerging concept is that combinations of gene therapy regimens with chemotherapy has synergistic antitumor effects. IFN- inhibits cell cycle progression as an S phase block; pretreatment of tumor cells with IFN- could significantly potentiate the cytotoxicity of cisplatin, 5-FU, paclitaxel and gemcitabine in cell cultures (150). Platinum-based chemotherapy enhances mutations in the p53 in the heterogenous cell population; transfer of the wild type p53 gene enhanced the sensitivity of chemoresistant cells to cisplatin and cisplatin-induced apoptosis (50).

A number of oncolytic adenoviruses designed to replicate selectively in tumor cells by targeting molecular lesions inherent in cancer, or by incorporation of tissue-specific promoters driving the early genes that initiate viral replication, are currently under clinical evaluation. Oncolytic adenovirus therapy shows the best results and achieves an enhanced tumoricidal effect when used in combination with chemotherapeutic agents such as cisplatin, leucovorin and 5-fluorouracil. Improvement of oncolytic adenoviruses is directed at molecular engineering tumor cell-specific binding tropism, selective modifications of viral early genes and incorporation of cellular promoters to achieve tumor-specific replication, augmentation of anti-tumor activity by incorporation of suicide genes, and manipulation of the immune response (151). Replication-activated adenoviral vectors have been developed to express a secreted form of - glucuronidase and a cytosine deaminase/uracil phosphoribosyltransferase. -glucuronidase activates the prodrug 9-aminocamptothecin glucuronide to 9-aminocamptothecin. The cytosine deaminase/uracil phosphoribosyltransferase activates the prodrug 5-fluorocytosine to 5-fluorouracil (5-FU) and further to 5-fluoro-UMP. The combination of this adenoviral vector with prodrug therapy enhanced viral replication and its spread in liver metastases derived from human colon carcinoma or cervical carcinoma in a mouse model (146).

Ototoxicity is a major dose-limiting side effect of cisplatin administration due to its propensity to induce destruction of hair cells and neurons in the auditory system. The trophic factor neurotrophin-3 protected spiral ganglion neurons (SGN) from cisplatin damage. Delivery of the neurotrophin-3 gene using a herpes simplex virus (HSV) amplicon vector and expression was able to partly bypass damage by cisplatin and attenuate the ototoxic action of cisplatin in organotypic cultures (152).

The human adenovirus type 5 (Ad5) early region 1A (E1A) proteins have been shown to have potent antitumor effects, due to their ability to reprogram oncogenic signaling pathways in tumor cells. The E1A gene of adenovirus functions as a tumor inhibitor by repressing oncogene transcription; E1A also modulates gene expression resulting in cellular differentiation and induces apoptosis in cancer cells. Finally, E1A sensitizes cancer cells to chemotherapeutic drugs such as etoposide, cisplatin, and taxol. An adenovirus vector deleted of all viral protein coding sequences with the exception of E1A reduced the proliferative capacity of the human lung adenocarcinoma cell line A549, the ability of these cells to form colonies in soft agarose and gave a 10-fold greater sensitivity to cisplatin (153).

Nine patients with recurrent and unresectable breast cancer and nine patients with head and neck cancer were treated intratumorally with a liposomal formulation of the E1A gene using 3 beta[N-(n',n'-dimethylaminoethane)-carbamoyl] cholesterol/ dioleoylphosphatidyl-ethanolamine. No dose-limiting toxicity was observed in the four dose groups (15, 30, 60, and 120 µg DNA/cm of tumor and a maximally tolerated dose was not reached. E1A gene transfer was demonstrated in 14 of 15 tumor samples tested, and down-regulation of HER-2/neu was demonstrated in two of the five patients who overexpressed HER-2/neu at baseline. In 16 patients evaluable for tumor response, 2 had minor responses, 8 had stable disease, and 6 had progressive disease (154).

Tumor cells have lost the function of p53 because of mutations mainly in the DNA-binding region of the molecule in over 50% of all human malignancies. Transfer of the wild type (wt) p53 gene was able to suppress tumor cell proliferation. The long-term follow-up of heavily pretreated patients with recurrent ovarian cancer by p53 gene replacement using the adenoviral vector SCH 58500 followed by multiple cycles of platinum-based chemotherapy has been evaluated by Buller and coworkers (155,156). The median survival of individuals who received multiple doses of the adenoviral p53 with chemotherapy was 12-13.0 months, compared to only 5 months for those treated with a single-dose of adenoviral p53; this compared favorably to the 16-month median survival for individuals treated with paclitaxel at the time of initial recurrence of this disease and was more than double the 5-month survival seen with palliative radiotherapy or paclitaxel failure. In spite of adenoviral-induced inflammatory changes, intraperitoneal adenoviral p53 treatment was safe as a 5-day regimen using 7.5×10^{13} adenoviral particles per dose per day followed by intravenous carboplatin/paclitaxel chemotherapy and resulted in a significant reduction of the serum tumor marker CA125 (155,156).

25. Conclusions

Platinum drugs remain a cornerstone of present day chemotherapy regimens not only for epithelial malignancies (lung, ovarian, bladder, testicular, head & neck) but also against a number of metastatic or advanced malignancies including cancers of the breast, melanoma, prostate, mesothelioma, nasopharyngeal, pancreatic, leiomyosarcomas and most other advanced cancers

(33). Their success mainly comes from the ability of platinum compounds to form bulky adducts on the DNA and crosslinks within the same strand or between the two strands. Understanding details on activation of signal transduction by cisplatin, carboplatin, oxaliplatin and most other platinum compounds leading to apoptosis, mostly responsible for the toxic side effects of platinum compounds, is likely to reveal novel strategies and improve combination therapies and efficacy. Inhibition of the JNK, ras, MAP kinase and other pathways by treatment of tumors with kinase inhibitors are bright prospects for new drugs to be used in this avenue.

Combination chemotherapy is important in most cancer treatment regimens because of synergy between chemotherapy drugs. The advantage using combinations of gemcitabine, topotecan, liposomal doxorubicin, and prolonged oral etoposide with platinum has been attributed to inhibition of DNA synthetic pathways involved in the repair of platinum-DNA adducts. Gemcitabine and cisplatin act synergistically, increase platinum-DNA adduct formation and induce concentration and combination dependent changes in ribonucleotide and deoxyribonucleotide pools in ovarian cancer cell lines (157). The combination of nedaplatin and irinotecan, a topoisomerase I inhibitor, showed synergistic interaction in cell cultures by concurrent exposure to both drugs; on the other hand, sequential exposure to the two drugs led only to additivity (158). Neither cisplatin nor carboplatin coadministration affected significantly the pharmacokinetics of etoposide on a randomized cross-over clinical trial involving 15 patients. Thus in this case the interaction between etoposide and platinum drugs is small and the clinical impact is unlikely to be significant (159). Many drug combinations involving platinum complexes have been explored, but those with taxanes are particularly noteworthy. Paclitaxel in combination with a platinum agent is now accepted as a standard component of first-line treatment for ovarian cancer, and produces improved survival (reviewed in 160). Recent clinical trials comparing concurrent chemotherapy and radiation with radiation alone in cervical cancer have shown that chemoradiation reduces the risk of death by 30-50% (32). In addition, recently randomized trials show an overall survival advantage of 30% for cisplatin-based chemotherapy given concurrently with radiation therapy (161). The molecular mechanism lies in the induction of strand breaks by the ionizing radiation that adds to platinum crosslinks and adds a formidable task to the DNA repair machinery.

Cisplatin induces oxidative stress and is an activator of stress-signaling pathways especially of the mitogen-activated protein (MAP) kinase cascades. Cisplatin adducts are repaired by the nucleotide excision repair (NER) pathway involving, among others, recognition of the damage by High Mobility Group (HMG) nonhistone proteins and mismatch repair proteins as well as ERCC-1, one of the essential proteins in NER. Defects in DNA mismatch repair produce low level resistance to cisplatin from the failure to recognize the cisplatin adduct and propagate a signal to the apoptotic machinery. Therapeutic interventions at all these molecular levels, either with gene transfer or with small molecules that interfere with these processes, would greatly affect the ability of cancer cells to cope with cisplatin damage. The discovery of novel platinum molecules could also lead to novel advancements in bypassing cisplatin resistance.

It is evident that different cancer cell types have different responses to platinum compounds. It is also evident that different types of sets of genes are active in particular cell types and that most platinum drugs display differences for damaging different genes depending on their transcriptional activity. For example active genes as well as their regulatory DNA have single-stranded regions and kinks or bends on their DNA as they interact with various classes of transcription factors or when they convert from the transcriptionally poised to the transcriptionally active state. Cisplatin has been shown to display a preference for single-stranded over double-stranded DNA as well as for chromatin regions either at the higher order domains or at the nucleosome level. In addition, different cancer cell types differ in their ability to repair incurring DNA damage (162,163). These differences between cancer cell types resulting in activation of different signal transduction or apoptotic pathways and in abundance of particular transcription factors or enzymatic activities arises from differential gene expression. These facts could explain the observed differences in behavior of tumor types to chemotherapy and chemoresistance.

Interleukins potentiate platinum drug cytotoxicity but can be used to improve bone marrow function. Cisplatin and IL-1 treatment induced a blockade at G1/S of the cell cycle, down-regulating c-myc gene and inducing p53-dependent apoptosis in ovarian carcinoma cells (164). Recombinant human interleukin-3 (rhIL-3) shortened the duration of chemotherapy-induced neutropenia and thrombocytopenia; concurrent administration of rhIL-3 and of a chemotherapy regimen for relapsed small cell lung cancer (vincristine, ifosfamide, mesna, and carboplatin on day 1 every four weeks) did not enhance myelotoxicity and improved bone marrow recovery (165). In addition, GM-CSF or erythropoietin are frequently used to improve bone marrow function and recovery from toxicity in cisplatin regimens.

Development of platinum drug resistance by tumors is a major clinical limitation. This is often linked to resistance to other chemotherapy drugs (doxorubicin, taxanes) from crosstalk of mechanisms involving import of these molecules across the cell membrane. The experimental strategies under investigation aimed at overcoming cisplatin resistance such as introduction of the functional p53 and p21 genes (22) usually mutated during carcinogenesis, or of genes that intervene with apoptotic pathways such bax (25), BclX$_L$ (42), bcl-2 (43) are likely to contribute to tumor shrinkage in combination with regimens using platinum drugs. For example, p53 is frequently mutated in late-stage cancer and the introduction of a functional wild-type p53 gene in gene therapy applications renders cancer cells sensitive to cisplatin (155,156 reviewed in ref. 51).

Novel avenues for anticancer therapeutic intervention include the development of hammerhead ribozymes or employment of oligonucleotides designed to cleave specific mRNAs and thus diminish the levels of a protein in a tumor cell. For example, suppression of the gene expression multidrug resistance 1 (MDR1) can reverse multidrug resistance to doxorubicin and etoposide (although not to cisplatin) in cell cultures (38). One could also inhibit factors that upregulate production of glutathione or other molecules involved in detoxification of cisplatin, express cell membrane molecules to enhance cisplatin import, diminish the repair activity or alter signaling pathways with the purpose of increasing cisplatin sensitivity in resistant tumors. Additional genes for intervention aimed at modulating cisplatin

chemoresistance may include the transcription factors AP-1 (40), UBF (41), mismatch repair genes (17,18), metallothionein (11), mdr1 (38,45), Pms2 (53), ERCC1 (24,74), XPA (74), homeobox genes (37), ERK (28,29), JNK (26,27), Akt (30), heme oxygenase (31), protein kinase phosphatases (48) and many other potential targets (reviewed in ref. 51). If such effects can be achieved by specific tumor targeting employing for example the liposome encapsulation technology implemented in Lipoplatin the effect would be very dramatic without interfering with normal tissues. The preferential tumor targeting via delivery of toxic or therapeutic anticancer genes in gene therapy applications using the type of liposomes used for liposomal encapsulation of genes and viruses (148) would bring a revolution in molecular medicine.

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