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Commentary

Resistance to Platinum Compounds: Mechanisms and Beyond

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PRECLINICAL MECHANISMS OF PLATINUM RESISTANCE

CISPLATIN has emerged as one of the most widely used antitumor cytotoxic agents. Whereas the electrophilic platinum coodination complexes formed as cisplatin is aquated react with a variety of nucleophiles and sites within the cell, the primary cellular target of cisplatin is DNA [1, 2]. Intrastrand, and to some extent interstrand, crosslinks which result from the interaction of cisplatin with DNA are responsible for the antitumor effects. The importance of cisplatin as a cornerstone of chemotherapy regimens for such potentially curable tumors as testicular, ovarian and small cell lung carcinoma is underscored by the numerous research efforts directed at the detection of the mechanisms responsible for tumor cell resistance to the drug. Efforts to identify the mechanisms of antineoplastic drug resistance could translate into new major therapeutic approaches for cancer. Delineation of clinical interventions which may circumvent tumor cell resistance and enhance the therapeutic index must remain an important goal in these research efforts.

Cellular resistance to antineoplastic agents has been classified as intrinsic or acquired. The same array of mechanisms may ultimately be responsible for the resistant phenotype in either setting. Intrinsic resistance is observed in tumors such as colon cancer, non-small cell lung cancer, renal cell cancer and melanoma, where even initial responses to chemotherapeutic intervention are unusual. This type of resistance may reflect the inherently increased occurrence of mutations to resistance within a tumor cell population [3]. The acquired resistance phenotype emerges after an initial tumor response to chemotherapy, and possibly results from the selective survival of residual resistant tumor cells after prior treatment.

The mechanisms of resistance to cisplatin which have been described are derived from studies in animal and human tumor models. A variety of mechanisms have been posited, including decreased drug accumulation, increased levels of intracellular protein and non-protein sulfhydryl molecules such as metallothionein (MT) and in some instances glutathione, and increased activity of the glutathione transferase system responsible for drug conjugation and neutralization (Table 1). Tumor cells which are resistant to cisplatin may also have increased capacity to recognize and repair drug-induced bidentate intrastrand DNA crosslinks. Masuda *et al.* [4] have demonstrated increased DNA repair in a human ovarian cell line which is 5-fold resistant to cisplatin. Treatment of the resistant tumor cells with aphidicolin, a DNA polymerase α inhibitor, resulted in partial reversal of the enhanced DNA repair activity of the resistant cell line, accompanied by a return of the IC_{50} for cisplatin toward that of the sensitive cell line [4]. The present study by Sekiya *et al.* describes the mechanisms of cisplatin resistance in the rat ovarian carcinoma cell

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Table 1. Mechanisms of CDDP resistance in tumor cell lines

Cell line (type)	Fold-resistance	Reported mechanism(s)	References
2008/DDP (human ovarian Ca)	3	↓ uptake no change thiols	[28]
2008/MT	4	no change uptake ↑ MT	[28]
COLO/MT (human ovarian Ca)	3	no change uptake ↑ MT	[28]
2780/CP (human ovarian Ca)	5	↑ GSH ↑ DNA repair	[4, 18]
Cis-Pt^r (ROT68/Ci) (rat ovarian Ca)	20	Uptake unchanged ↑ GSH ↑ DNA repair	[5]
L1210/DDP (murine leukemia)	18	↓ uptake ↓ a.a. transport	[29]
L1210/DDP5	48	↓ uptake ↑ GSH	[30]
L1210PtR4	13.5	↓ uptake	[31]
L1210/CP	44	↑ MT	[24]
P388/PtR4 (murine leukemia)	24	↓ uptake GSH unchanged	[31]
K562DDP (human CML)	7	no change uptake ↓ a.a. transport	[32]
HA-C4; HA-C6 (hamster fibroblast)	2.5	↓ uptake	[27]
SCC-25/CP (human head/neck Ca)	7–20	↓ uptake GSH unchanged ↑ MT ↑ GST ↓ DNA crosslinking	[20, 24]
NIH 3T3/CP	4–8	activated H ras gene	[33]
G3361/CP (human melanoma)	6	↑ MT	[24]
SW2/CP (human SCLC)	4	↑ MT	[24]

Abbreviations used: MT = metallothionein; GSH = glutathione, GST = glutathione-*S*-transferase; CML = chronic myelogenous leukemia; SCLC = small cell lung carcinoma; a.a. = amino acid.

line ROT68/Cl. The Cis-Pt^r subline demonstrated increased unscheduled DNA synthesis after exposure to cisplatin, a result of increased activity of the DNA repair system [5]. There was no alteration in cellular uptake of cisplatin and glutathione content was increased. Interestingly, the Cis-Pt^r cells had an abnormality of chromosome 13 (13q+). The authors note that karyotypic abnormalities have also been described in other cisplatin-resistant cells. The exact relationship between these chromosomal changes and the expression of the resistance phenotype remains to be determined.

POTENTIAL CLINICAL MEANS TO CIRCUMVENT PLATINUM RESISTANCE

Clinically relevant resistance to cisplatin has been associated with *in vitro* situations in which 3–5-fold higher drug concentrations are required to achieve cytotoxic effects equal to those produced in sensitive tumor cells or nonmalignant cells. Given the steep dose–response curve observed with cisplatin and carboplatin, methods of treatment which permit significant dose escalation may offer promise in efforts to overcome tumor resistance to platinum compounds. Several strategies have been developed to decrease the toxicity to normal host tissues which accompanies higher individual or cumulative doses of platinum therapy. Some of the supportive interventions described here have yielded favorable results in early clinical studies.

Experimental studies in human ovarian cancer cell lines have confirmed that the dose–response relationship is steep in these cell lines [6], justifying attempts to overcome resistance *in vivo* by escalation

of drug levels. Clinical studies have shown improvement in the response rate of advanced refractory ovarian cancer when the cisplatin dose was increased. Ozols *et al.* [6] administered a cisplatin dosage of 200 mg/m^2/cycle with 3% saline to induce chloruresis. A 32% response rate was reported in patients who received high dose cisplatin as second- or third-line treatment when they no longer responded to their prior therapy or had recurrent disease after an initial response to conventional dose cisplatin. Substantial toxicities were observed, including transient acute renal failure and neurotoxicity which was dose-limiting. All patients treated using this high-dose cisplatin regimen developed some sensory neuropathic symptoms. The most severe symptoms occurred in 37% of the patients, and included gait abnormalities and proprioceptive losses. This neurotoxicity limits the number of courses of high-dose cisplatin which can be administered.

Carboplatin [CBDCA, *cis*-diammine-1,1-cyclobutane-dicarboxylatoplatinum (II)] has also been used in high-dose therapy of refractory ovarian carcinoma [7]. Patients who had received prior cisplatin chemotherapy were treated with carboplatin at 800 mg/m^2/cycle with 0.9% saline hydration. A response rate of 27% was reported. Patients who had previously developed progressive tumor concurrent with cisplatin therapy did not respond to the high-dose carboplatin regimen, consistent with clinical cross-resistance between the two platinum compounds in ovarian carcinoma. The predominant toxicity observed in this study was myelosuppression. Additional phase I attempts to escalate carboplatin dose to 1600–2400 mg/m^2/cycle [8, 9] have defined myelosuppression and hepatotoxicity as potential dose-limiting toxicities in adult patients. No phase II information at these higher doses is currently available in ovarian cancer. Efforts to incorporate high dose carboplatin into combination chemotherapy regimens are justified to capitalize on the dose–response curve and the acceptable patient tolerance in the single-agent high dose carboplatin trials.

Continued attempts to escalate platinum dosage have led to incorporation of a variety of sulfhydryl-rich nucleophiles into experimental treatment regimens, in hope of achieving protection from toxicity. The earliest dose-limiting toxicity encountered for cisplatin was nephrotoxicity. In addition to the previously mentioned chloruresis using 3% saline, renal tubular protection from platinum damage has also been demonstrated using sodium thiosulfate, presumably from reaction of the thiolate anion with reactive and toxic platinum metabolites produced after cisplatin administration. If thiosulfate is concentrated in the urine and, therefore, neutralizes cisplatin in the kidney to a greater extent than in the plasma, simultaneous administration of cisplatin and thiosulfate may result in an incease in the therapeutic index for cisplatin. Pfeifle *et al.* [10] have assembled pharmacokinetic data which show that peak plasma cisplatin concentrations and areas under the concentration–time curves (AUC) for cisplatin at 202.5 mg/m^2 plus thiosulfate are at least twice those measured in patient receiving cisplatin alone at a dose of 100 mg/m^2. Nephrotoxicity was reduced, but not prevented, by concomitant use of thiosulfate, while the incidence of other adverse effects such as nausea, vomiting, neurotoxicity, ototoxicity and myelosuppression was comparable to other high-dose cisplatin studies without the protective agent. The suggestion of increased patient exposure to platinum with selective renal protection by thiosulfate must be questioned, however, as no tumor responses were documented in the Pfeifle study of high-dose cisplatin and thiosulfate. *In vivo* studies of concomitant systemic thiosulfate and cisplatin administration also reveal some expansion of the therapeutic dose range of cisplatin at the cost of significant reductions in the maximum increase in lifespan (% ILS) of mice bearing L1210 leukemia [11, 12]. However, *intraperitoneal* cisplatin therapy combined with systemic thiosulfate can reduce nephrotoxicity of high-dose cisplatin without compromise of the intraperitoneal antitumor effect [13]. Further investigations of the effect of thiosulfate on the therapeutic index of cisplatin are warranted.

Sodium diethyldithiocarbamate (DDTC) is a heavy metal chelator. DDTC reacts with and removes platinum from all sites except DNA crosslinks, and has demonstrated protective effects on the kidney, bone marrow and GI tract when given after cisplatin in animal models [14]. Rothenberg *et al.* [15] conducted a Phase II study to determine whether DDTC could protect against the myelosuppressive effects of high-dose carboplatin without compromise of the antitumor activity. When DDTC was administered 3 h after carboplatin (800 mg/m^2/cycle), to patients with refractory ovarian carcinoma, a response rate of 19% was observed. No additional hydration was administered and there was no report of nephrotoxicity or neurotoxicity attributed to carboplatin therapy. Unfortunately, severe myelosuppression was encountered when DDTC was added to carboplatin therapy, with a trend towards even more severe depression of leukocyte and platelet counts than in the previously described high-dose carboplatin trial at 800 mg/m^2/cycle. In addition, the toxicity of DDTC, including anxiety and autonomic dysfunction, limits the potential for this thiol compound to yield significant platinum chemoprotection.

WR-2721 [*S*-2-(3-aminopropylamino)ethyl phosphorothioic acid] has emerged from screening efforts to define cysteine compounds which selectively protect normal tissues from the toxic effects of radiation, alkylating agents and cisplatin chemo-

therapy [16]. WR-2721 is metabolized via hydrolysis to an intermediate compound (WR-1065) capable of chelating platinum. Since WR-2721 pretreatment does not seem to confer tumor cell protection from cytotoxic effects, clinical research efforts [16] have attempted to increase the dosage of cisplatin administered, while preventing concomitant drug toxicity. Preliminary phase I and II trials using WR-2721 pretreatment with cisplatin doses of 120–150 mg/m^2 suggest that the dose-limiting toxicity of WR-2721 is hypotension, and that WR-2721 may potentiate the antitumor activity of cisplatin. Improvement in the response rate of refractory tumors such as melanoma has been reported [17]. Subsequently, larger cooperative trials have been initiated to assess the response rate, response duration and survival in patients receiving cisplatin ± WR-2721 in melanoma and metastatic breast carcinoma. Further studies will assess the effect of WR-2721 on platinum compound pharmacokinetics, given preliminary data which suggest that WR-2721 may decrease the plasma half-life of cisplatin. Additional evaluation of the protection afforded in carboplatin therapy by pretreatment with WR-2721 is also being pursued.

Attempts at reversal of the cisplatin-resistance phenotype *in vitro* and in animal models include studies using buthionine sulfoximine (BSO). BSO inhibits cellular production of glutathione, presumably in a nonselective fashion. Since glutathione inhibits the binding of aquated cisplatin to DNA [14], cells with elevated levels of glutathione may demonstrate resistance to cisplatin due to decreased DNA crosslink formation or sequestration of the platinum compounds at other cytosolic sites. Some cisplatin-resistant tumor cells, such as the human ovarian carcinoma cell lines reported by Hamilton *et al.* [18], have elevated glutathione content, and may have the resistance phenotype reversed if total cellular glutathione levels are reduced by BSO treatment. Several other investigators suggest that a decrease in tumor cell glutathione content using BSO does not reverse the cisplatin resistance *in vitro* [19, 20]. The *in vitro* data do not clearly define whether glutathione decreases the toxicity and antitumor effect of platinum compounds by direct interaction with the parent drug or by reaction with metabolites of cisplatin. Unfortunately, BSO, in doses which decreased tissue glutathione levels, proved toxic in animal studies [21]. Dogs treated with BSO and cisplatin demonstrated enhanced renal toxicity and lethality, thereby limiting the ability to increase the cisplatin therapeutic index in the clinical setting of resistance [22]. It may be more fruitful to combine BSO treatment with non-nephrotoxic platinum compounds such as carboplatin, if additional studies validate the benefit of decreasing cellular glutathione levels as a means to reverse platinum resistance.

Bohm *et al.* [23] have reported that glutathione itself can be added to cisplatin–cyclophosphamide chemotherapy for ovarian carcinoma with no compromise in therapeutic efficacy but with a decrease in occurrence of severe myelosuppression and nephrotoxicity. The cisplatin dose used in the study was 90 mg/m^2. Confirmation of this chemoprotection is still needed.

It may be possible to increase the therapeutic index of cisplatin by normal tissue protection mediated by increased cellular metallothionein (MT) content. MT is a ubiquitous sulfhydryl-rich protein containing 30% cysteine residues. Multiple inducers of MT synthesis have been identified *in vivo* including stress, dexamethasone, heavy metals, endotoxin, ionizing radiation and alkylating agent exposure [24]. As a result of *in vivo* studies, Satoh *et al.* have suggested that MT can be induced in normal tissues such as kidney and bone marrow by pretreatment with the heavy metal compound bismuth subnitrate [25]. Tumor-bearing animals receiving concomitant bismuth and cisplatin were protected from cisplatin nephrotoxicity and myelosuppression, without compromise of the antitumor effect of cisplatin. MT levels were documented to increase in the kidney but were unchanged in the tumor cells after bismuth administration. Additional studies are necessary to optimize the methods for induction of MT and to ascertain the degree of tissue-specificity of the induction. In addition, if measures to selectively reduce tumor cell MT content are developed, it may also prove possible to modulate the cisplatin resistance phenotype through depletion of the elevated levels of MT shown to be a factor in several preclinical models of cisplatin resistance [24].

Additional mechanisms to modulate cisplatin resistance have been suggested in studies of nucleoside membrane transport inhibitors such as dipyridamole. Dipyridamole has been demonstrated to partially reverse cisplatin-resistance in a human ovarian cell line, apparently through increases in cisplatin uptake and enrichment of the intracellular concentration of the active, aquated species of cisplatin [26]. The potentiation of toxicity of cisplatin which may result from enhanced uptake of the drug by normal cells as well as tumor cells remains to be defined *in vivo*.

The synergy of hyperthermia and cisplatin chemotherapy has been demonstrated *in vitro*. Dose enhancement of cytotoxic effect was similar in both cisplatin-sensitive and -resistant cells at 39 and 41°C, but with escalation of temperature to 43°C, the dose enhancement ratio in the resistant cells was more pronounced [27]. Hyperthermia increased cisplatin accumulation by about 2-fold in the cisplatin-resistant cells. It is also encouraging that

cross-resistance between hyperthermia and cisplatin has not been demonstrated *in vitro*. The temperatures and cisplatin concentrations used in these studies are achievable clinically without the need for marked dose escalation. Therefore, such combined modality therapy may warrant further clinical study with cisplatin or carboplatin, especially as an approach to regional therapy of particular clinical problems, such as abdominal carcinomatosis in the setting of refractory ovarian adenocarcinoma.

Thus, an array of mechanisms for tumor cell resistance to cisplatin have been described. It is reasonable to conclude that acquired cisplatin resistance is the result of combinations of changes at various sites in the tumor cell, from the membrane level to the excision of DNA adducts. The challenge lies in the transition from preclinical models of resistance to rational clinical attempts at modulation of the resistance phenotype. In addition, development of platinum analogs to which tumor cells are not cross-resistant will expand the spectrum of applications of this important class of antineoplastic agents.

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