Molecular Pathways: The Immunogenic Effects of Platinum-Based Chemotherapeutics

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Abstract
The platinum-based drugs cisplatin, carboplatin, and oxaliplatin belong to the most widely used chemotherapeutics in oncology, showing clinical efficacy against many solid tumors. Their main mechanism of action is believed to be the induction of cancer cell apoptosis as a response to their covalent binding to DNA. In recent years, this picture has increased in complexity, based on studies indicating that cellular molecules other than DNA may potentially act as targets, and that part of the antitumor effects of platinum drugs occurs through modulation of the immune system. These immunogenic effects include modulation of STAT signaling; induction of an immunogenic type of cancer cell death through exposure of calreticulin and release of ATP and high-mobility group protein box-1 (HMGB-1); and enhancement of the effector immune response through modulation of programmed death receptor 1-ligand and mannose-6-phosphate receptor expression. Both basic and clinical studies indicate that at least part of the antitumor efficacy of platinum chemotherapeutics may be due to immune potentiating mechanisms. Clinical studies exploiting this novel mechanism of action of these old cancer drugs have been initiated. Here, we review the literature on the immunogenic effects of platinum, summarize the clinical advances using platinum as a cytotoxic compound with immune adjuvant properties, and discuss the limitations to these studies and the gaps in our understanding of the immunologic effects of these drugs. Clin Cancer Res; 20(11); 2831–7. ©2014 AACR.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

CME Staff Planners' Disclosures
The members of the planning committee have no real or apparent conflict of interest to disclose.

Learning Objectives
Upon completion of this activity, the participant should have a better understanding of the immunologic effects of platinum drugs that have been recently uncovered and the biologic rationale for new combination treatments incorporating immunotherapy and platinum drugs.

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Background
Biochemical mechanism of action of platinum compounds
Platinum-based compounds are some of the oldest, most potent, and widely used cytotoxic drugs in cancer treatment.

Metastatic germ cell tumors can be cured with platinum-containing regimens, and many types of locoregional advanced cancer can be cured in a substantial amount of patients when treated with platinum in combination with radiotherapy and/or surgery (1). In the case of metastatic disease, platinum-based drugs are widely used as palliative treatment in many cancers (1). Three platinum compounds are used worldwide: cisplatin, carboplatin, and oxaliplatin. Cisplatin is approved by the U.S. Food and Drug Administration (FDA) for use in bladder, cervical, ovarian, testicular cancer, non–small cell lung cancer (NSCLC), mesothelioma, and head-and-neck squamous cell carcinoma (HNSCC). Carboplatin is approved for treatment of ovarian and lung cancer, and oxaliplatin for colon and pancreatic cancer (1). It is evident that platinum-based compounds are an essential component of contemporary cancer intervention strategies.
Nevertheless, their precise mechanism of action remains not fully understood. Platinum compounds enter the cell and undergo hydrolysis, thereby losing the chloride or oxalate ions and giving rise to the reactive mono-aqua and di-aqua derivatives (Fig. 1A). Reactive platinum molecules bind to nucleophilic groups containing oxygen, nitrogen, or sulfur donors (2). These groups are omnipresent in a cell, in amino acid side chains of proteins, and the purine bases of RNA or DNA (3). The binding to DNA, thereby forming platinum–DNA adducts, is thought to be the mechanism of action. This addsuct interfere with transcription and DNA replication, initiating a DNA-damage recognition response that results in apoptosis (Fig. 1A; refs. 1, 4–6).

The assumption that DNA is the most important target for platinum is based on several findings, including (i) a correlation between the levels of platinum–DNA adducts and treatment efficacy (7–9); (ii) enhanced sensitivity to cisplatin-induced cell death of DNA repair-deficient Escherichia coli bacteria compared with wild-type bacteria (10); and (iii) the amount of platinum bound to intracellular proteins or RNA was initially deemed insufficient to affect their function (11–13).

However, other targets could be involved in the mechanism of action of platinum drugs. Of the covalently bound platinum in a cell, only 5% to 10% is bound to DNA (14). At an equitoxic dose of cisplatin versus oxaliplatin or carboplatin, the level of DNA adduct formation is 10 times higher with cisplatin, suggesting that a mechanism other than DNA adduct formation may be involved in tumor cell cytotoxicity (15, 16). One of these potential mechanisms is RNA binding, as platinum accumulates to 4- to 20-times higher levels in cellular RNA compared with DNA in Saccharomyces cerevisiae (17).
addition, cisplatin causes atypical cross-links in structurally complex RNAs, thereby preventing reverse transcription, indicating that the RNA binding may interfere with communicating sequence information \cite{18, 19}.

Cellular proteins form another non-DNA target of platinum drugs. Platinum has been reported to bind to several proteins, including ubiquitin, Hsp90, G-actin, and other cytoskeletal proteins \cite{2, 20–23}. For some of these, the binding resulted in conformational changes and subsequent altered biologic function \cite{21–23}.

The strongest evidence that non–DNA-binding properties of platinum drugs can be involved in their cytotoxicity was provided by Bose and colleagues \cite{24}, who synthesized a novel platinum compound that was highly active, displaying in vitro cytotoxicity similar to that of cisplatin, but did not bind to DNA at all. The mechanism that mediates its cytotoxicity is still unknown \cite{24}. In summary, platinum drugs bind to several non-DNA target molecules in the cell and recent findings indicate that these non-DNA interactions could play a role in the efficacy of these anticancer drugs.

**Clinical–Translational Advances**

**Modulation of STAT protein signaling by platinum compounds**

Because evidence was accumulating that cancer chemotherapeutics could have beneficial effects on the immune system \cite{25}, we investigated their effects on dendritic cells (DC) in vitro. Surprisingly, platinum drugs, but not other chemotherapeutic agents, strongly enhanced T-cell activation by DCs. This was caused by downregulation of the cell-inhibitory molecule programmed death ligand (PD-L) 2, which is regulated by the IL-4/STAT6 signaling pathway \cite{26, 27}. Binding of interleukin (IL)-4 to its receptor normally leads to phosphorylation of STAT6 in the cytoplasm followed by dimerization of phosphorylated STAT6 monomers and translocation to the nucleus, and culminating in initiation of transcription of STAT6 target genes, such as PD-L2. Treatment with platinum caused a loss of STAT6 phosphorylation, which resulted in the downregulation of PD-L2 \cite{26}, not only on DCs but also on tumor cells. PD-L2 downregulation on tumor cells resulted in enhanced recognition by T cells. The significance of STAT6 inactivation for clinical practice was demonstrated in a retrospective study. Patients with STAT6-expressing HNSCC had a poorer outcome after treatment with radiotherapy, compared with patients with STAT6-negative tumors. However, if the STAT6-positive tumors were treated with radiotherapy and cisplatin, the patients had a significantly better recurrence-free survival rate, compared with patients with STAT6-negative tumors. These results hint at the possibility that STAT6 expression by tumors contributes to an immunosuppressive microenvironment that results in a poorer prognosis, unless attacked by platinum \cite{28}.

Although we now have a better understanding of the effect of platinum drugs on STAT6, the effects of these drugs on other members of the STAT family remain inconclusive. STAT3 activation is inhibited by suprapharmacologic levels of cisplatin \cite{29}, and both STAT3 expression and phosphorylation are inhibited by developmental platinum compounds CPA-1 and 7 \cite{30–32}. In contrast, increased phosphorylation of STAT6 and STAT1 after in vitro treatment with suprapharmacologic concentrations of cisplatin has been reported as well \cite{33, 34}. Intriguingly, the latter study found that knockdown of STAT1 decreased the cytotoxic effect of cisplatin \cite{34}. Whether these differences in inhibition or activation of STATs relate to different cell types used in the indicated studies is unclear.

**Platinum-induced immunogenic cell death**

Experiments in immunocompetent versus immunodeficient mice demonstrated that certain chemotherapeutic compounds are more effective in the presence of an intact immune system. These compounds, including oxaliplatin, induce a combination of tumor cell stress and death that can induce tumor-specific immune responses \cite{35–37}.

On a cellular level, three requirements have to be met for this immunogenic cell death (ICD; Fig. 1B; refs. 35, 37–39). First, calreticulin exposure serves as a signal for DCs to engulf the dying cell, whereas release of ATP and high mobility group protein-1 (a nuclear protein that regulates DNA transcription) mediates DC activation and maturation via signaling to their respective receptors, purinoceptor P2RX7 and pattern recognition receptor TLR4. Calreticulin, a calcium-binding chaperone that prevents misfolded proteins from being exported by the endoplasmic reticulum (ER), is exposed as oxaliplatin induces an ER stress response that triggers the translocation of calreticulin to the plasma membrane \cite{39}. The exact oxaliplatin-dependent trigger is still unknown but is linked to the non–DNA-binding effects of oxaliplatin as treatment of cells without nuclei, cytoplasts, still induces calreticulin exposure \cite{38}. The second requirement, ATP released by dying cancer cells, functions as a powerful chemotactic find-me signal, recruiting DCs and macrophages to the tumor site \cite{40}, where they engulf fragments of calreticulin-exposing cells. In addition, ATP stimulates the maturation of DCs, leading to increased expression of costimulatory molecules CD40, CD80, and CD86 \cite{41, 42}. The third requirement for ICD is the release of HMGB-1 from the nucleus, which binds to TLR4 on DCs, leading to improved cross-presentation and increased proinflammatory cytokine secretion \cite{35, 43}.

Surprisingly, cisplatin does not induce ICD, despite its presumed identical mechanism of action to that of oxaliplatin. This is attributed to the lack of calreticulin exposure after cisplatin treatment, as both compounds induce ATP and HMGB-1 release. However, the clinical efficacy of cisplatin could still be potentiated by ICD as cisplatin is often administered concurrently with radiotherapy. Radiotherapy is a potent inducer of calreticulin exposure \cite{44}, and recent studies show that combining cisplatin with compounds that induce calreticulin exposure leads to full-blown ICD \cite{36, 45}. 
It is important to note that the studies on ICD used transplanted tumor cells in syngeneic murine hosts. Transplanted tumor models inherently differ from naturally arising tumors in terms of their establishment, growth, microenvironment, and potential release of antigens, all of which can influence the antitumor immune response. Mammary tumors were equally susceptible to treatment with platinum in mice with or without an intact adaptive immune system when oncogene-driven tumors were used, as opposed to transplantable subcutaneous tumors (46). It is possible that the absence of a preexisting T-cell response in these spontaneous tumors precludes the reactivation of a pool of effector memory T cells (47).

Although the induction of ICD by platinum drugs under specific conditions is clear, it remains unclear how much ICD contributes to their clinical efficacy. Analyses of 338 patients with colorectal cancer that participated in a randomized phase III trial comparing upfront oxaliplatin-based combination chemotherapy versus sequential chemotherapy showed that patients with a loss-of-function allele for TLR4 displayed poorer progression-free survival (PFS) and overall survival (36). These data indicate that ICD induction by oxaliplatin may indeed contribute to its clinical efficacy, although it is clear that other effects play a role as well.

Other immune-potentiating effects of platinum drugs
In addition to inducing ICD, platinum drugs also sensitize tumor cells to CTL-mediated attack. Cisplatin treatment of murine lymphoma and colon cancer cells caused upregulation of mannose-6-phosphate (M6P) receptors, which rendered tumor cells sensitive to granzyme-B killing (48). As a result, a small number of tumor-specific CTLs were able to mediate a strong antitumor response that was not limited to antigen-expressing tumor cells. In addition, pretreatment of tumor cells with oxaliplatin increases MHC class I expression, thereby counteracting immune evasion and promoting DC maturation and T-cell proliferation (49).

Clinical studies exploiting the immunogenic momentum of platinum
An important question is how to further exploit the immunogenic momentum of platinum to increase its anti-cancer efficacy. A novel route being explored is the combination of platinum and immune checkpoint blockade. Checkpoint molecules are cell-surface receptors on T cells that upon triggering induce a cascade of signaling events that restrain T-cell proliferation and killing capacity (50). Many checkpoint molecules have been discovered, and antibodies that block their activation are being investigated in oncology. A CTL antigen (CTLA)-4 blocking antibody, ipilimumab, was FDA-approved for use in metastatic melanoma, and antibodies against PD-1 or its ligand, PD-L1, are in advanced clinical development in melanoma and NSCLC (50). There is a good rationale to combine platinum compounds with checkpoint-blocking antibodies: Platinum can provide ICD (in case of oxaliplatin or cisplatin-based chemoradiation), tumor cell sensitization to CTL lysis, and downregulation of PD-Ls, while the checkpoint blockade unleashes the immunocidal potential of activated T cells (50). A randomized phase II study in NSCLC showed an increased PFS rate when ipilimumab was combined with carboplatin/paclitaxel compared with chemotherapy alone (51); a phase III trial is currently ongoing. Also, in small-cell lung cancer, the combination of ipilimumab with platinum chemotherapy resulted in an improved, immune-related PFS rate in a randomized phase II trial (52). Importantly, in both studies, the additive effect was dependent on the timing of the drugs, which could be due to a temporarily negative effect on T-cell proliferation by chemotherapy. This could also explain conflicting results in animal mesothelioma models investigating anti-CTLA-4/cisplatin combinations (53, 54).

Recently, investigators from a phase I study combining platinum doublets with the PD-1 blocking antibody nivolumab reported an acceptable toxicity profile and encouraging response rates (53), but more mature data are needed before conclusions can be drawn. It will be interesting to know whether there is an additive effect of combination therapy over checkpoint blockade alone, a strategy that was not investigated in the anti-CTLA-4 studies, but is included in the anti-PD-1 studies. This will provide insights into the true immunogenic effects of platinum drugs in patients with cancer.

Vaccination studies have shown that T-cell responses induced by vaccines are not hampered by coadministration of platinum, and in fact can be augmented (56–58); however, studies with significant effects on clinical endpoints are currently lacking.

Finally, selected drugs may restore the capacity to induce ICD when combined with the non–ICD-inducing platinum drug cisplatin (45, 59). For example, cardiac glycosides, which are used in the treatment of congestive heart failure, are able to induce ICD. These drugs were shown to extend the PFS and overall survival of patients with colon cancer treated with non–ICD-inducing chemotherapy but not in patients treated with oxaliplatin (59, 60). However, these data have not been confirmed in prospective trials. Thus, although the immunogenic effect of platinum drugs is currently being explored in the clinic, their true immune-modulating effect in patients has not been established yet.

Remaining questions and future perspective
As discussed, platinum drugs react with a wide array of macromolecules, including DNA, RNA, and proteins, all possibly contributing to their clinical activity. Perhaps the unequal activity of platinum in many different types of cancer is due to this wide mode of interaction on multiple levels. Nevertheless, there is a degree of specificity involved, as demonstrated for STAT proteins and the induction of ICD. However, the exact relevance of these pathways in platinum cytotoxicity is unclear.

The clinical efficacy of platinum drugs could be dependent on the immune system via mechanisms regulated by...
the non–DNA-binding effects of these drugs. Although our understanding of the role of the immune system in platinum efficacy is far from complete, it is clear that platinum can modify the immune response during both the induction and the effector phase (Fig. 2). The induction of ICD leads to the recruitment of DCs to the tumor. These DCs engulf dying cancer cells and mature, while PD-L1 and PD-L2 on the DCs, increasing their T-cell activation potential. The mature DCs migrate to the lymph node, where they prime naïve, tumor-specific T cells into effector cytotoxic T cells, which migrate to the tumor microenvironment. Platinum drugs inactivate STAT6 in the tumor cells, leading to decreased PD-L2 expression, resulting in enhanced recognition and killing by the tumor-specific T cells. Platinum induces upregulation of M6P receptor on tumor cells, which leads to enhanced tumor cell lysis by granzyme-B secreted by the activated T cells. The increased M6P receptor expression also induces lysis of cancer cells that are not in direct contact with tumor-specific T cells.

To obtain a complete picture of the complex, multilevel mechanism of action of platinum and accurately assess the relevance of platinum-mediated immune modulation in the clinical efficacy of these drugs, prospective clinical studies are needed. Furthermore, a number of questions still need to be resolved. First, which of the interactions, with DNA, RNA, or proteins, is most relevant for the clinical efficacy of platinum compounds? Second, which proteins and signaling pathways are affected by platinum compounds? Besides the signaling pathways that lead to ICD, there are other candidates including STAT pathways; is only STAT6 inhibited by platinum drugs or other STATs as well? Third, do these drugs transform the immunosuppressive microenvironment into an immunostimulatory site? And finally, platinum drugs are often administered in combination with other chemotherapeutics; how do these compounds influence the immunologic effects?

Elucidating these important questions will greatly increase our understanding of the mechanism of action of these drugs. Furthermore, this previously neglected characteristic of platinum could be useful in the context of combination therapies, potentiating the effect of targeted therapies or immunotherapy.
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