Cisplatin Nephrotoxicity

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Cisplatin remains a major antineoplastic drug for the treatment of solid tumors. Its chief dose-limiting side effect is nephrotoxicity, which evolves slowly and predictably after initial and repeated exposure. The kidney accumulates cisplatin to a higher degree than other organs perhaps via mediated transport. Functionally, reduced renal perfusion and a concentrating defect characterize its nephrotoxicity, whereas morphologically necrosis of the terminal portion of the proximal tubule and apoptosis predominantly in the distal nephron characterize its effects on cell fate. Among the earliest reactions of the kidney to cisplatin is the activation of the MAPK cascade and molecular responses typical of the stress response. Repression of genes characteristic of the mature phenotype of the kidney, especially those serving transport function of the kidney, is also prominent. Metabolic responses, cell cycle events and the inflammatory cascade seem to be important determinants of the degree of renal failure induced by cisplatin. Manipulation of these responses may be exploited to reduce its toxicity clinically.

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CISPLATIN, AMONG THE MOST commonly used antineoplastic agents for the treatment of solid tumors, is also perhaps the best-studied antineoplastic nephrotoxin. Long-term survivors have provided the opportunity to follow cisplatin nephrotoxicity over longer periods of time than has been possible with other agents. Preclinical studies identified nephrotoxicity as cisplatin’s major dose-limiting side effect early,1 and initial protocols not using aggressive hydration before administration of cisplatin produced severe and frequently irreversible renal failure.2 Other significant side effects, such as ototoxicity and severe nausea and vomiting, also may limit its use.

PATHOGENESIS OF CELL INJURY
Renal Uptake and Metabolism of Cisplatin

The kidney accumulates and retains platinum to a greater degree than other organs and is the principal excretory organ for injected cisplatin.3 In the rat, the kidney excretes the drug rapidly within the first hour of its administration by a process consisting predominantly of glomerular filtration, with a minor component of secretion.4-6 There is no evidence of tubular reabsorption, suggesting that the kidney accumulates cisplatin by peritubular uptake.5 The uptake of cisplatin by the kidney is dependent on temperature and the normal consumption of oxygen and can be inhibited by drugs that participate in the organic base transport system, suggesting that at least some portion of renal cisplatin uptake is facilitated.5 Consistent with the view that cisplatin nephrotoxicity may be linked to how the kidney transports cisplatin is the high cisplatin content of proximal straight tubules after 195m[Pt] cisplatin injection,7 the principal site of necrosis after cisplatin.8 Changes in the distal nephron consisting of mitochondrial swelling and nuclear pallor, if not frank necrosis, also have been described.2 The glomerulus is spared of obvious morphologic changes. Apoptosis predominantly in the distal nephron also has been described in cisplatin nephrotoxicity.9

Excreted platinum is predominantly cisplatin, but once cisplatin traverses the cell membrane it is converted to several species.10 Once gaining access into the cell, cisplatin is thought to undergo aquation reactions in which the labile chloride ligands are replaced by water molecules, resulting in a positively charged and highly reactive electrophilic product.11 Recent evidence suggests that some of these products may be important to the nephrotoxicity of cisplatin.12 The primary lethal lesion produced by cisplatin in cancer cells is its intrastrand binding to adjacent purine bases,13 which alters the secondary structure of DNA, inhibiting its template function and inhibiting DNA replication.14,15 This lesion is not produced by the transplatin isomer, which is neither antineoplastic nor nephrotoxic. Whether the nephrotoxicity of cisplatin also depends on such damage to DNA is unknown. Cisplatin also may damage the kidney by depletion of critical sulfhydryl centers,16 including glutathione, and provoke damage to the cell in much the same manner as oxidant stress.
MOLECULAR RESPONSES TO CISPLATIN TREATMENT

Role of the Mitogen Activated Protein Kinases in Cisplatin Nephrotoxicity

Cisplatin induces a prominent immediate early gene response in the kidney including expression of the stress-related proteins c-jun and junD.17 Cellular stress, including DNA-damaging chemotherapeutic drugs, activates 2 related signaling pathways: the stress-activated protein kinase, also known as Jun N-terminal kinase (JNK), and the extracellular regulated kinase (ERK)-mitogen-activated protein kinase.18 Targets of stress-activated protein kinase/JNK include c-Jun and JunD components of the activated protein 1 (AP-1) transcription factor, which becomes activated after genotoxic exposure,19,20 suggesting a role of AP-1–dependent transcription in cisplatin-induced cell death. Cisplatin-mediated activation of JNK and concomitant cell death are related because expression of a dominant-negative expression vector of the JNK kinase MKK4 blocked cell death.21 Similarly, cells derived from c-jun knockout mice were more resistant to cisplatin-induced cell death than normal cells.22 However, the role of JNK in cisplatin-induced death of renal proximal tubular cells is yet to be determined.

Role of Caspase Activation in Cisplatin-Induced Cell Death

Cisplatin induces apoptosis of renal proximal tubular cells (LLCPK) in vitro via mitochondria-dependent and -independent pathways,23 in part by activation of caspase-3.24 Caspase 3–dependent and –independent pathways to cell death coexist in cisplatin-induced cytotoxicity and p53 also may play a role independent of these pathways as well.23–25 Thus, the pathway to execution of cell death is multifactorial. It is interesting to note that DNA damage has been shown to cause translocation of JNK to the mitochondria and phosphorylation and inactivation of the antiapoptotic Bcl-XL protein.26 Nevertheless, the relationship between the JNK pathway and the caspase cascade or mitochondrial cytochrome c release has not been explored. A role for ERK activation in cisplatin-induced apoptosis also has been suggested by studies in rabbit proximal tubule cells.27 Also, depending on the dose of cisplatin, renal tubular cells undergo either necrosis or apoptosis;28 this phenomenon is characterized by differential regulation of caspases.

Cytokines and Cisplatin-Induced Cell Death

Cisplatin administration significantly up-regulates several cytokines and chemokines (tumor necrosis factor α (TNFα), transforming growth factor β, RANTES, MIP-2, monocyte chemotactic protein-1) in the kidney.29 Increased TNFα leads to the recruitment and accumulation of inflammatory cells, which can directly injure surrounding renal tissue. Anti–intercellular adhesion molecule I antibodies reduce cisplatin toxicity further, suggesting a proinflammatory role of cisplatin in its nephrotoxicity.30 Interestingly, TNFα itself provokes oxidant-dependent damage to cells so that the possibility that the TNFα-related toxicity is direct and unrelated to recruitment of inflammatory cells cannot be ruled out until its toxicity is explored in leukocyte-depleted animals.

Cell Cycle Events in Cisplatin Nephrotoxicity

Genotoxic damage either by intercalation of DNA or by oxidant-induced damage may be an important component of the stress imposed by cisplatin. Cisplatin provokes increased expression of the cyclin-dependent kinase inhibitor p21WAF1/CDPI, which inhibits completion of the cell cycle and promotes DNA repair. Interestingly, mice with deletion of the p21 gene are more susceptible to cisplatin nephrotoxicity,9 suggesting that it may be critical to the repair of cisplatin-induced damage to renal cells. This is discussed in detail by Price et al in this issue of Seminars.

Physiologic Hallmarks of Cisplatin Nephrotoxicity

Cisplatin predictably lowers glomerular filtration rate in a dose-dependent manner,32 even after single drug exposure. The onset of renal failure is gradual, usually occurring 3 to 5 days after its administration. Early proteinuria is mild (<500 mg/d), as is glycosuria. Enzymuria is common, even in the mildest forms of acute renal failure. In a few patients, significant urinary electrolyte wasting may be provoked by cisplatin,33 including severe sodium, divalent action, and phosphate wasting, but this presentation is uncommon and primarily is seen with high-dose therapy. Most common is the gradual onset of nonoliguric renal failure with water excretion in excess of solute.8 In micropuncture experiments, the site of altered wa-
ter reabsorption is beyond the late distal tubule, probably in the apparently morphologically intact collecting duct.\textsuperscript{8} In rats, the cause of the decrease in glomerular filtration is afferent vasoconstriction and possibly an altered ultrafiltration coefficient, before evidence of tubule obstruction.\textsuperscript{33} The precise pathogenesis for these changes in segmental water transport and vascular resistance remains unknown.

Cisplatin also produces hypomagnesaemia\textsuperscript{34} in a large percentage of patients receiving the drug. Although it may occur acutely, it usually is observed after repeated exposure over longer periods of time.\textsuperscript{35} It often is found when additional drugs with significant potential for magnesium wasting are used in conjunction with cisplatin, such as nephrotoxic antibiotics such as gentamicin or amphotericin,\textsuperscript{36} or when cisplatin is used in combination with other chemotherapeutic agents.\textsuperscript{35} In the normomagnesemic animal ingesting a diet containing adequate magnesium, cisplatin does not induce hypomagnesemia. However, in the rat kept on a magnesium-deficient diet, cisplatin induced a defect in maximal reclamation of an administered magnesium load, suggesting that hypomagnesemia is a consequence of diets deficient in magnesium in combination with a mild defect in magnesium reabsorption.\textsuperscript{37} Serum calcium level is usually normal in such cases.

Repeated cisplatin administration has been shown to reduce glomerular filtration rate chronically in a dose-related manner.\textsuperscript{38} Patients receiving up to 850 mg in multiple courses had a 9% reduction in hippuran clearance, whereas patients receiving more than 850 mg of cisplatin had a 40% reduction in glomerular filtration rate over a 5-year period.\textsuperscript{38} This study also found significant potentiation of cisplatin nephrotoxicity in those patients receiving cisplatin combined with radiotherapy.

Protective Measures

The wide therapeutic application of cisplatin depends not only on its clinical efficacy but also on the development of strategies that diminish its nephrotoxicity. The most commonly used protective measure is to establish a solute diuresis.\textsuperscript{39} A commonly applied protocol is to establish a diuresis before (12-24 h) administering cisplatin, which then is given in isotonic saline by a 3-hour infusion, followed by infusion of isotonic saline for 24 hours after the drug is infused. Cisplatin usually is administered in daily divided doses for 5 days until the maximum dose is reached, usually not to exceed 120 mg/m\textsuperscript{2} body surface area. Beyond this dose, cisplatin provokes an unacceptable degree of renal failure even in the presence of a diuresis. The mechanism of the protection is not known. Although the concentration of cisplatin is lower in the urine, neither the tissue concentration of the drug nor the degree of cytotoxicity is altered by such maneuvers.\textsuperscript{39}

Additional maneuvers have been attempted to reduce cisplatin nephrotoxicity. Diethyldithiocarbamate, which has been shown to compete for platinum binding to DNA,\textsuperscript{40} has shown promise experimentally\textsuperscript{41} but has been much less successful in human trials owing to its toxicity and failure to modify cisplatin ototoxicity.\textsuperscript{42} Inorganic thiophosphates such as WR2721 (amifostine) have been shown to be effective in preventing renal failure even after repeated exposure.\textsuperscript{43} The mechanism of protection may involve promotion of better DNA repair and synthesis by amifostine\textsuperscript{44} and/or by the release of free thiols after dephosphorylation with alkaline phosphatase, which then act as free radical scavengers and metal binding centers.\textsuperscript{45}

Manipulation of the signal transduction and molecular pathways provoked by cisplatin may provide an opportunity in the future to influence cisplatin-induced nephrotoxicity. A balance between the JNK and ERK pathways was proposed to dictate the cellular decisions of death and survival: transient activation of JNK when also accompanied by activation of pro-survival ERK or AKT pathways may not be associated with cell death. Indeed, inhibition of JNK by dominant-negative mutants proved to be protective of cisplatin-induced injury.\textsuperscript{21} Pharmacologic inhibition of the cisplatin-induced ERK activity in ovarian carcinoma cells caused enhanced cisplatin cytotoxicity.\textsuperscript{46} Inhibition of the cisplatin-induced Akt/PKB pathway enhanced activation of caspases-3 and -9 in proximal tubular cells.\textsuperscript{24} Inhibition of the ERK or Akt cascades diminishes cisplatin-induced phosphorylation of BAD (a proapoptotic member of the bcl-2 family) and thus, sensitizes cells to cisplatin.\textsuperscript{47} At least in ovarian carcinoma cells, inhibition of ERK sensitized cells to cisplatin injury\textsuperscript{48,49} as well. Inhibitors of TNF\textsubscript{a} production significantly improved cisplatin-induced renal dysfunction. Similarly, TNF\textsubscript{a}-deficient mice were resistant to cisplatin nephrotoxicity.\textsuperscript{30} Thus, manipulation of...
the signal transduction pathways in such a way as to increase survival and decrease death-inducing pathways seems to be a reasonable approach to ameliorating cisplatin nephrotoxicity. More work on in vivo systems will help resolve which pathways serve each of these functions.

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