Acute Nephrotoxicity of Cisplatin: Molecular mechanisms

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Abstract

The nephrotoxic drugs have been responsible for about 20% of AKI episodes in inpatients and outpatients. The cisplatin nephrotoxicity is a major limiting factors in 20% of patients who have received the drug, triggering injuries in renal tubular epithelialcells. Cisplatin toxicity is determined by the target tissue and cells accumulation besides the interaction with various subcellular structures and macromolecules. Cisplatin accumulates and interferes with the functioning of different organelles such as mitochondria, lysosomes, endoplasmic reticulum, nuclei and cell membranes, causing inflammation and cell death. This review aims to define the pathophysiology and biochemistry of the cisplatin nephrotoxicity, reviewing the main molecular mechanisms that lead to tubular cisplatin toxicity.

Keywords: acute kidney injury, acute toxicity, cisplatin.

Introduction

Nephrotoxic drugs are responsible for approximately 20% of the episodes of acute kidney injury (AKI) in hospital and outpatient settings. Among elderly individuals, the incidence of drug-induced nephrotoxicity can be as high as 66%. Cisplatin (cis-diamminedichloroplatinum II - CDDP) is an oncologic medication included in most chemotherapy regimens for solid or hematologic tumors. Cisplatin's antineoplastic properties were accidentally discovered by biophysicist Barnett Rosenberg, but nephrotoxicity was found to be a significant limiting factor for up to 20% of the patients on the drug. While most alkylating antineoplastic agents cause DNA damage exclusively to fast growth cells, cisplatin can also cause considerable damage to the relatively quiescent cells of the proximal renal tubule. The use of cisplatin is limited by tumor cell resistance and adverse effects such as nephrotoxicity, ototoxicity, neurotoxicity, and high emetic risk.[1-3]

Cisplatin's nephrotoxicity is attributed to two main factors: high concentrations of cisplatin in the kidneys and adverse impacts on the renal transport system. Cisplatin is predominantly excreted by the kidneys; biliary and intestinal excretion is minimal. However, in renal excretion the drug accumulates in the kidneys and even non-toxic blood levels may reach toxic levels in the kidneys. Cisplatin concentrations in tubular epithelial cells are five times greater than in blood. Cisplatin-induced renal toxicity is dose-dependent, and thus limits the possibility of increasing dosages; consequently, treatment effectiveness may be impaired. Toxic effects occur primarily in the proximal tubule, particularly in S3 segment of the tubular epithelial cells; glomeruli and distal tubules are affected subsequently. Renal function deterioration is seen in 25% to 35% of the patients treated with a single dose of cisplatin. Decreases of 20% to 40% in glomerular filtration can be observed 10 days after drug intravenous administration, and are followed by increased levels of creatinine, reduced glomerular filtration rates (GFR), hypomagnesemia, and hypokalemia.[3,4]

The delayed effects of cisplatin on renal function have not been fully understood, but cisplatin therapy is believed to result in subclinical or permanent glomerular filtration reduction in some patients.[5]

The pathophysiology of cisplatin nephrotoxicity has been studied for three decades. Recent studies attempted to understand the cellular and molecular mechanisms of nephrotoxicity, linking it to local accumulation of cisplatin inside the proximal tubule, intracellular conversion of the drug into toxic metabolites, and damage to multiple pathways. The pathophysiological mechanism of cisplatin-induced tubular damage is complex and involves a number of interconnected factors, such as accumulation of cisplatin mediated by membrane transportation, conversion into nephrotoxins, DNA damage, mitochondrial dysfunction, oxidative stress, inflammatory response, activation of signal transducers and intracellular messengers, and activation of apoptotic pathways.[3-5]

Pathophysiology and biochemistry of cisplatin nephrotoxicity

The pathophysiology of cisplatin toxicity can be grouped into four types of injury. However, knowledge of the links between pathophysiological events is crucial to understanding renal syndromes caused by cisplatin. Cisplatin can cause tubular toxicity, which often manifests through water and electrolyte disorders and acute renal failure by tubular necrosis; small and medium artery vascular damage; glomerular injury, which is less frequent than other nephropathies; and interstitial injury secondary to prolonged exposure to cisplatin, which may progress to chronic kidney disease.[5]

Next, we will focus on the main biochemical mechanisms of tubular cell toxicity caused by cisplatin, the drug's main driver of kidney injury.

Tubular injury by cisplatin

The proximal tubule loses the epithelium responsible for favoring the flow of substances into its cells. This process involves the formation of concentrated urine, which also leads to increased levels of potential toxins in the tubular fluid and passive diffusion of toxins into tubular cells.[5]

Cisplatin is a neutral low molecular weight compound filtered freely in the glomerulus and almost entirely retrieved in urine. In this process, the drug penetrates the tubular cells and reaches high concentrations in the proximal tubular cells of the inner renal cortex and outer medulla (S3 segment), the sites most dramatically affected by cisplatin. Dose-dependent injury can also occur in the distal tubule and collecting duct system.[6,7]

The proposed injury pathway is as follows: 1) accumulation of cisplatin mediated by the transport pathway; 2) metabolic conversion of cisplatin into nephrotoxins and accumulation in kidney cells; 3) DNA injury; 4) cell transport system alterations; 5) mitochondrial dysfunction 6) oxidative and nitrosative stress; 7) inflammatory response; 8) activation of mitogen-activated protein kinase (MAPK); and 9) activation of apoptotic pathways.

Transporter-mediated cisplatin accumulation

Cisplatin enters renal tubular cells by passive diffusion or transporter-mediated facilitated diffusion, leading to disproportionate drug accumulation. A basolateral organic cation transporter (OCT) has been linked to penetration of cisplatin in renal tubular cells and as a determining factor in drug pharmacokinetics and adverse effect severity, including nephrotoxicity.[8] Three isoforms of OCT have been identified in humans. OCT2 is the main organic cation transporter in the kidneys, OCT1 in the liver, and OCT3 is particularly expressed in the placenta. In rats, OCT1 was the main transporter type observed in the proximal convoluted tubule (S1) and proximal straight tubule (S2), with low levels of expression in the outer medullary proximal straight tubule (S3); OCT2 was expressed mainly in the S2 and S3 segments. OCT2 is a critical transporter in cisplatin penetration and cytotoxicity in the proximal tubules and affects accumulation of the drug in the kidneys. Recent studies have shown that OCT1/OCT2-deficient mice were protected from cisplatin-induced tubular damage. Interestingly, cisplatin does not interact with OCT1, which could help explain the drug's organ and cell-specific toxicity. Additionally, the high-affinity copper transporter (CTR1) is also expressed in the basolateral face of the proximal

tubules. CTR1 downregulation in kidney cells *in vitro* decreased cisplatin uptake and cytotoxicity, suggesting that CTR1 is an important mechanism in the absorption of cisplatin in renal cells. The role of CTR1 in cisplatin nephrotoxicity *in vivo* has not been examined.[5,8,9]

Metabolic conversion of cisplatin to a nephrotoxin and drug accumulation in kidney cells

When administered intravenously, cisplatin quickly diffuses to tissues and binds to plasma proteins, given the strong reactivity of platinum complexes with amino acid thiol groups such as cysteine. Approximately 90% of the platinum in the blood binds to albumin and other plasma proteins, which leads to the inactivation of a large number of cisplatin molecules. The chloride ligands in cisplatin must be displaced before the drug binds to DNA. The extracellular concentration of sodium chloride is of approximately 100 mM, whereas in cells the concentration of chloride ranges between 20 and 30 mM, thus allowing cisplatin hydration to occur; subsequently, water molecules replace one or two chloride ligands, resulting in the formation of [Pt (H_2O) Cl (NH_3) 2] + and [Pt (H_2O) 2 (NH_3) 2] 2 + cations. These cations originate positively charged molecules which easily react with nuclear DNA to form covalent bonds with purine bases, especially in the N7 position, resulting in 1,2-intra-chain cross-links strongly correlated with cisplatin-induced genotoxic effects.[3,10]

Positively charged platinum ions are more toxic in kidney cells than in the parenteral system, as they bind to components of DNA, RNA, and proteins. The cross-links between DNA and cisplatin impair the replication and transcription functions, leading to cell cycle arrest and apoptosis.[3,10]

Wainford *et al.*[11] suggested that intracellular enzyme gamma-glutamyl transpeptidase (GGT) plays a role in the metabolism of cisplatin as a nephrotoxin, as this enzyme cleaves the cisplatin-glutathione conjugate to a toxic metabolite.

DNA damage

Cisplatin is cytotoxic as it forms cross-links within and between kidney genomic DNA chains. The degree of platination has been more commonly associated with the entry of cisplatin in cell nuclei secondary to drug accumulation. The platinum-DNA bond generates adducts or new compounds that activate various cellular responses, including signaling of DNA damage, cell cycle checkpoints, DNA repair, and cell death.[12]

Hydrated forms of cisplatin easily react with nuclear DNA, forming covalent bonds with purine bases, primarily in the N7 position, resulting in 1.2-intra-chain cross-links strongly correlated with the genotoxic effects of cisplatin. The cross-links between DNA and cisplatin lead to impaired replication and transcription, resulting in cell cycle arrest and, eventually, apoptosis.[13] The target of apoptosis causing DNA damage is mediated by tumor suppressor protein p53, which activates pro-apoptotic and suppresses anti-apoptotic genes. Dividing cells are particularly sensitive to DNA cell damage, and cisplatin's antineoplastic activity has been attributed mainly to the formation of DNA adducts. However, some authors have suggested that the formation of nuclear DNA adducts may not be the sole determining factor in the pharmacological effect of cisplatin; indeed, mitochondrial DNA may be the most common binding target for cisplatin due to its poor repair capabilities. In adult males, proximal tubule cells do not divide. Therefore, the formation of DNA adducts may not have a major role in cisplatin nephrotoxicity. In addition to nuclear and mitochondrial DNA, cisplatin affects other cell components such as RNA, proteins, and phospholipids. Other mechanisms have been associated with the nephrotoxic effects of cisplatin on healthy renal cells. Oxidative damage and inflammation may explain such effects on other cell components associated with cisplatin renal toxicity. Several lines of evidence indicate that cisplatin nephrotoxicity is associated mainly with mitochondrial reactive oxygen species (ROS).[4,12-14]

It has been suggested that cisplatin conjugates with reduced glutathione (GSH) in the liver reach the kidneys as a platinum-GSH conjugate, which is cleaved primarily to a toxic metabolite mostly by gamma-glutamyl transpeptidase (GGT), an enzyme located in the brush border of the renal proximal tubule. The formed metabolite is highly reactive with thiol/platinum compounds

that interact with macromolecules and eventually lead to renal cell death.[11] This biotransformation has been proposed as a means to prevent the formation of nephrotoxic metabolites and, thereby, minimize the nephrotoxicity of cisplatin. It has been shown that mice deficient in GGT were resistant to the nephrotoxic effects of cisplatin. Additional studies with rats have shown that inhibition of GGT with acivicin protected them from cisplatin nephrotoxicity. The participation of other enzymes such as aminopeptidase N (AP-N), renal dipeptidase (RDP), and cysteine S-conjugate beta-lyase (C-S lyase) in this toxic pathway.

The following sequence was proposed: after platinum-GSH conjugates are secreted into the lumen of the proximal tubule, they are cleaved by GGT forming a cysteine-glycine conjugate and are then cleaved by cell surface aminopeptidases (AP-N, RDP) to form a cysteine conjugate, which is then reabsorbed by the proximal tubule and ultimately metabolized by the C-S lyase into toxic thiol compounds resulting in nephrotoxicity. The inhibition of C-S lyase with aminooxyacetic acid protected mice treated with 15 mg/kg of cisplatin. In another study, the inhibition of enzymes AP-N, RDP, and C-S lyase did not protect rats given 10 mg/kg of cisplatin and/or rats treated with 6 mg/kg from cisplatin-induced nephrotoxicity.[11,15]

Cell transport system alterations

Cisplatin-induced nephrotoxicity is characterized by renal proximal tubular cell dysfunction. Cisplatin interferes with the transport of water and renal tubular cell nutrients. Transport is mediated by sodium pumps in the apical and basolateral face of the cells, such as Na/K/ATPase, Na-K-2Cl cotransporter, and type III Na/H exchanger, and water-permeable channels including aquaporins 1, 2, and 3. Cisplatin inhibited the activity of transporters in the brush border both in both *in vivo* and *in vitro* models. Cisplatin-induced injury may interfere with the integrity of the cytoskeleton and cell polarity, leading to changes in hydrogen, potassium, magnesium, and calcium ions and contributing to lower ion reabsorption rates in the proximal and distal tubules and increased excretion of these ions in urine. Furthermore, loss of the tubular epithelial cell barrier and/or junctions between viable cells in cisplatin-induced tubular injury could also force the glomerular filtrate to flow back into blood circulation, producing an apparent decrease in GRF.[16]

Mitochondrial dysfunction

Several lines of evidence suggest that cisplatin accumulates in the mitochondria of renal cells, hampering mitochondrial bioenergetics, increasing the generation of reactive oxygen species (ROS), decreasing the absorption of calcium in the mitochondria, and causing the release of pro-apoptotic factors which ultimately lead to renal tubular cell death.[17]

There is evidence that mitochondrial DNA and other mitochondrial targets are perhaps more important than nuclear DNA damage in mediating cisplatin-induced cell death. Cisplatin is hydrolyzed to generate a positively charged metabolite that preferentially accumulates within the negatively charged mitochondria. Thus, the sensitivity of cells to cisplatin seems to correlate well with mitochondrial density and mitochondrial membrane potential. This observation may explain the particular sensitivity of the renal proximal tubule to toxicity, since this segment has one of the highest mitochondrial densities in the kidney. A comparison of cisplatin-sensitive and cisplatin-resistant ovarian cancer cells revealed lower mitochondrial membrane potential and less mitochondrial DNA damage in resistant cells, indicating mitochondrial DNA may be more sensitive than nuclear DNA to cisplatin-induced injury for having less effective DNA repair mechanisms. Taken together, these observations point to mitochondrial DNA as an important target of cisplatin toxicity.[14,18]

Energy production in the mitochondria is also disrupted by cisplatin and may contribute to nephrotoxicity. Fatty acids are the main source of energy for the proximal tubule, the site in the kidneys most affected by cisplatin-induced injury. Cisplatin inhibits the oxidation of fatty acids in the kidneys of rats and in proximal tubule cells in culture by decreasing the expression of peroxisome proliferator-activated receptor alpha (PPAR-alpha) mediated by the genes involved in the utilization of cell fatty acids.[19]

Cisplatin also affects mitochondrial respiratory complexes and their function. *In vitro* exposure of cultures of proximal tubule cells to cisplatin inhibits mitochondrial complexes I to IV of the respiratory chain and, as a result, leads to decreased intracellular levels of ATP. *In vivo* administration of cisplatin also resulted in mitochondrial dysfunction as evidenced by decreases in membrane electrochemical potential, substantial reductions in mitochondrial calcium uptake, and depletion of mitochondrial antioxidant defense systems.[18,19]

Oxidative and nitrosative stress

Substantial evidence indicates that oxidative stress is involved in renal injury secondary to cisplatin administration. ROS production, depletion of antioxidant systems, and stimulation of renal accumulation of lipid peroxidation products have been listed as the main mechanisms associated with cisplatin-induced nephrotoxicity. These mechanisms cause the activation of oxidative metabolism by stimulating the production of ROS by impaired mitochondria, including superoxide anions (O_2), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH); they may also impair antioxidant defense mechanisms such as GSH, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Nitrosative stress is involved in cisplatin-induced renal damage. Studies revealed that the cellular effects of ROS are amplified by high production levels of nitric oxide (NO), possibly as a result of induced synthesis of nitric oxide synthase (iNOS), leading to the continuous formation of peroxynitrites (ONOO-), which contribute to cisplatin-induced renal damage by reacting with superoxide anions. The increased production of reactive oxygen and nitrogen species after cisplatin administration results in significant damage to cell structure and function, including lipid peroxidation, protein nitration, enzyme inactivation, and DNA breaks. Consequently, this phenomenon leads to cell dysfunction and signaling for the activation of both apoptotic and cell survival pathways, causing kidney damage and cell death.[20-23]

Cisplatin may induce ROS formation in microsomes via the cytochrome P450 system (CYP). *In vitro* and *in vivo* tests revealed that CYP was an important source of catalytic iron for the generation of ROS during treatment with cisplatin. In CYP2E1-null mice, cisplatin-induced accumulation of ROS and kidney damage were attenuated. Despite the role of oxidative stress in cisplatin nephrotoxicity, the critical molecular targets of ROS in renal tubular cells are still unknown. Given their largely reactive nature, ROS may attack and modify multiple molecules present in cells such as lipids, proteins, and DNA, and produce cellular stress. ROS also appear to be involved in the activation of important signaling pathways in cisplatin-induced nephrotoxicity, including apoptotic pathways. These observations suggest that ROS may be seen as early signs partially responsible for the activation of various signaling pathways that culminate in kidney failure, renal injury, and cell death in the event of cisplatin-induced nephrotoxicity.[24,25]

In addition to injury, oxidative stress stimulates kidney cells to produce a cytoprotective response. This is best illustrated by heme oxygenase 1 (HO-1). HO-1 is a redox-sensitive microsomal enzyme that catalyzes the degradation of heme oxygenase to biliverdin, iron, and carbon monoxide. HO-1-deficient rats were significantly more sensitive to cisplatin-induced kidney damage as compared to wild controls. HO-1 overexpression significantly reduced *in vitro* cisplatin-induced apoptosis. The molecular basis for the cryoprotective effects of HO-1 is not entirely clear, but the proposed mechanisms include the degradation of the pro-oxidant portion of heme oxygenase, generation of antioxidant bilirubin, and the production of cryoprotective carbon monoxide. In fact, a study showed that carbon monoxide can significantly improve renal injury induced by cisplatin *in vitro* and *in vivo*. Future research involving the role of HO-1 and its products may not only provide a mechanistic understanding of cisplatin-induced renal injury, but also lead to the identification of best renal protectants.[26,27]

Several experimental models demonstrated the renal protection offered by antioxidants such as dimethylthiourea (DMTU), melatonin, selenium, vitamin E, and N-acetylcysteine, to name a few. However, renal protection has not been reported for chemotherapy patients given cisplatin. Interestingly, natural antioxidant products may

decrease the levels of ROS in the kidneys without decreasing the effectiveness of cisplatin. Although the active ingredients in these natural products are not known, if the renal protection effect they offer is proven true in humans, they may potentially be used in therapeutic applications.[3,4,18]

Inflammatory response

Evidence suggests inflammatory mechanisms are strongly linked to the pathogenesis of cisplatin nephrotoxicity. Cisplatin activates phosphorylation and the subsequent translocation of nuclear factor *kappa* B (NF-κB) transcription factor to the nucleus with the degradation of inhibitory IκBα protein. Activation of NF-κB promotes the transcription of specific genes encoding inflammatory mediators and producing immune, proliferative, anti-apoptotic, and inflammatory responses.[18] This event leads to increased expression of tumor necrosis factor alpha (TNF-α) in kidney tubular cells, an important cytokine involved in systemic inflammation and acute-phase response induced by the administration of cisplatin. TNF-α can trigger tubular cell death and tissue damage directly through TNF receptor type 1 (TNFR1) and indirectly by mounting a strong inflammatory response through TNF receptor type 2 (TNFR2). Furthermore, TNF-α/TNFR2 signaling contributes to cisplatin nephrotoxicity and may enhance the pro-apoptotic effects arising from the activation of TNFR1. TNF-α is known to coordinate the activation of a large network of proinflammatory cytokines such as interleukin-1, 4, 6 (IL-1β, IL-4, IL-6), transforming growth factor-β1 (TGF-β 1) and monocyte chemotactic protein-1 (MCP-1), stimulated after the activation of RANTES (regulated on activation, normal T cell expressed and secreted) cytokines. Additionally, TNF-α induces the expression of adhesion molecules, including intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin, promoting an inflow of inflammatory cells in tissues. TNF-α is produced locally by specific kidney cells and not by cells derived from the bone marrow or the immune system infiltrating the kidneys in cases of cisplatin-induced nephrotoxicity.[28] Thus, the infiltration of inflammatory cells may act as reservoirs of inflammatory cytokines and chemokines, which may enhance the cytotoxic effects of cisplatin and lead to loss of kidney function and fibrosis through the generation of ROS, NO, and proinflammatory cytokines.[29]

In summary, TNF-α appears to play a key role in the downstream regulation of the inflammatory response triggered by cisplatin. However, the upstream signaling responsible for the production of TNF-α remains uncertain. Zhang *et al.*[30] proposed a role for toll-like receptors (TLR), a family of receptors considered as the innate first line of defense, in the production of cytokines and the onset of renal dysfunction in cases of cisplatin-induced nephrotoxicity. The authors demonstrated that TLR4 is essential in the initiation of the intra-renal inflammatory response associated with cisplatin-induced nephrotoxicity.

Activation of mitogen-activated protein kinases (MAPKs)

The signaling system of mitogen-activated protein kinases (MAPKs) consists of multiple highly conservative serine/threonine protein kinase activity pathways activated by extracellular signals and cell regulatory processes such as proliferation, differentiation, migration, apoptosis, and survival. The production of c-Jun N-terminal kinases (JNKs) and p38 MAPK is induced by cellular stress, inflammatory response, and apoptotic pathways initiated by a variety of stressful biological, physical and chemical stimuli. The cascade of extracellular-signal-regulated kinases (ERKs) is induced mostly by cell death and cell survival growth factors. Authors have described different activation patterns in the three main MAPK pathways (ERK, JNK, and p38) using *in vitro* and *in vivo* experimental models of cisplatin nephrotoxicity.[3,4]

The events downstream of p38 MAPK activation which lead to the synthesis of TNF-alpha in cisplatin-induced renal inflammation have been described. However, it has been reported that the activation of MAPK p38 in neutrophils stimulated by lipopolysaccharides and in smooth muscle cells of blood vessels leads to Iκβ (an inhibitor of NF-κβ) degradation, consequently promoting the activation and migration of NF-κβ to the nucleus, producing pro-inflammatory cytokines including TNF-α. Nonetheless, some of these inflammatory

mediators, including TNF-α, form a loop feedback which induces the phosphorylation and degradation of inhibitory protein Iκβα and the transcription of genes of inflammatory mediators.[20]

Transforminggrowthfactor-β(TGF-β),monocyte chemotactic protein-1 (MCP-1), intercellular adhesion molecules (ICAM) and HO-1 have been implicated in cisplatin nephrotoxicity. Significant up-regulation of TNF-α, TGF-β, RANTES, macrophage inflammatory protein 2 (MIP-2), MCP-1, TCA3 (T-cell activation-3), IL-1b, and ICAM-1 was found in kidneys of animals treated with cisplatin. Increased levels of interleukin-1b (IL-1b) have been associated with inflammatory caspases (IL-1b-converting enzyme or ICE), one of which being caspase-1, which activate other cytokines such as IL-18 and IL-6 and promote neutrophil infiltration. Inhibition of IL-1b, IL-18, IL-6 or neutrophil infiltration in the kidneys is not enough to prevent kidney injury induced by cisplatin, but caspase-1-deficient mice are protected from apoptosis and acute tubular necrosis. This could be explained by the involvement of caspase-1 in the apoptotic pathway, which in addition to participating in the inflammatory process, could also activate caspase-3 and induce renal tissue apoptosis.[3,4,18]

Apoptotic pathway activation

Two major apoptotic pathways have been implicated in cisplatin nephrotoxicity, including (i) the intrinsic pathway involving cell organelles such as the endoplasmic reticulum and mitochondria, and (ii) the extrinsic pathway, also called death receptor pathway, which involves the activation of death receptors in response to binding membrane receptors. Both pathways lead to the activation of specific proteases called executioner caspases (caspase-3 and caspase-7), resulting in characteristic morphological signs of apoptosis which include membrane blistering, cell shrinkage, and DNA fragmentation.[4,31]

The mitochondrial or intrinsic pathway has emerged as a key factor for renal tubular cell death in experimental models of cisplatin-induced nephrotoxicity. BCL-2 family pro-apoptotic proteins (BAX and BAK) function as 'molecular integrators' to the mitochondrial pathway, and *in vivo* models have been used to document their role in cisplatin-induced apoptosis. After exposure to signs of cell death, pro-apoptotic proteins BAX and BAK undergo structural modifications and alter the integrity of the mitochondrial membrane to cause the release of apoptogenic factors such as cytochrome C (caspases activator) and apoptosis-inducing factor (AIF), a caspase-independent cell death promoter. Despite the release of cytochrome C in response to cisplatin, the inhibition of cytochrome C mediated by the activation of caspase provided only partial protection from cisplatin-induced apoptosis, suggesting a role for AIF in cell death. Two other important mechanisms have been described in cisplatin nephrotoxicity, in addition to the downstream regulators of the apoptotic pathways and proteins of the BCL-2 family: signal transduction promoters such as protein kinases (MAPK, PI3K, and PKB/Akt) and transcription factors (NF-kB and p53). The role of the p53 protein has been recognized as critical for the induction of apoptosis in cisplatin nephrotoxicity. Studies suggest that the activation of p53 may be an early sign of apoptosis induced by the presence of cisplatin in renal tubular cells, as it promotes the activation of caspase-2 and the mitochondrial release of AIF, two major cell death pathways, also demonstrating that DNA damage is induced by the translocation of AIF and correlated with p53.[3,31,32]

The endoplasmic reticulum (ER) can also initiate apoptosis directly or by interfering with the mitochondrial pathway. Caspase 12, located on the cytosolic face of the endoplasmic reticulum which is activated by stress, is the initiator of the ER pathway. Ca^{2+}-independent phospholipase A2 is another protein associated with the ER implicated in cell death. In renal tubular cells treated with cisplatin, this protein may act downstream of p53 and upstream of caspase-3. The extrinsic pathway initiated by the binding of cell death receptors through plasmatic membrane ligands leads to the recruitment and activation of caspase-8 and caspase-10, which activate caspase-3 to possibly recruit the mitochondrial pathway. The main ligands of cell death include Fas and TNF-α and their corresponding receptors (TNFR 1 and 2).[4,31]

Death receptor-mediated apoptosis induced by cisplatin has been observed in human proximal tubular epithelial cells and associated with increased

expression of Fas and Fas ligand in renal tissues. On the other hand, TNFR1 contains a conservative death domain after binding to TNF-α that may trigger complex caspase activation and apoptosis, subsequently. In contrast, TNFR2 lacks the death domain and therefore may not be directly involved with the early stages of apoptosis.[29,32]

Conclusion

Nephrotoxicity is a serious limiting adverse effect for patients with cancer on cisplatin that stems from the transport of cisplatin in renal epithelial cells, nucleus and mitochondrial DNA injury, activation of cell death by multiple pathways, and onset of intense inflammatory response. Despite potential therapeutic targets, interventions in animal models have produced only partial protection against nephrotoxicity. Additionally, the impact of such interventions on the effectiveness of cisplatin chemotherapy has not been adequately examined. Clinical trials have proposed new combined preventive strategies to target various molecular mechanisms connected to cisplatin nephrotoxicity.

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