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## Cisplatin-Associated Nephrotoxicity and Pathological Events

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### Abstract

Cisplatin (*cis*-diamminedichloroplatinum(II)) is an effective chemotherapeutic agent, and is successfully used in the treatment of a wide range of tumors. Despite its effectiveness as an anti-tumor drug, nephrotoxic side effects have significantly restricted its clinical use. Tubular epithelial cell deletion following cisplatin treatment is a major cause of renal injury. Oxidative stress significantly contributes to cisplatin-associated cytotoxicity, and use of antioxidants could counteract such cytotoxic effects of cisplatin. The renal microenvironmental changes following cisplatin treatment is a complex process and could be broadly categorized into three main pathological events, which at times might overlap: initial cytotoxic events, inflammatory events and fibroproliferative events. Stress responses and heat shock proteins generated following cisplatin treatment are actively involved in the initiation and progression of these events. In this article, we will briefly summarize factors involved in various phases of cisplatin-induced renal injuries.

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### Introduction

*Cis*-dichlorodiaminoplatinum (II), cisplatin, is one of the most widely used antineoplastic drugs. Cisplatin is an inorganic complex formed by an atom of platinum surrounded by chlorine and ammonia atoms in the *cis* position of a horizontal plane. One of the possible mechanisms by which cisplatin accumulates in the cells is by a carrier-mediated processes, through probenecid-sensitive organic anion transporters; the chloride ions are displaced by hydrolysis, resulting in the formation of highly reactive, charged platinum complexes. Probenecid restricts renal secretion of anionic drugs through inhibition of the organic

**Table 1.** Partial list of tumors where cisplatin has been used as an antitumor drug

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Adrenocortical tumor
Bladder tumor
Brain tumor
Breast tumor
Cervical tumor
Endometrial cancer
Gastrointestinal tumor
Germ cell tumor
Gynecological sarcoma
Head and neck tumor
Hepatoblastoma
Lung cancer, small cell
Malignant melanoma
Neuroblastoma
Non-Hodgkin's lymphoma
Osteosarcoma
Ovarian tumor
Testicular tumor
Thyroid tumor

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anion transport system(s). Coadministration of probenecid has shown to decrease renal excretion of various drugs including cidofovir, ciprofloxacin and cisplatin [1]. Probenecid could interfere with tubular secretion of cisplatin, and thereby could increase cisplatin toxicity. On entry into the cell, the platinum compounds cross-link with DNA; this binding of platinum to complexes of DNA apparently disrupts and unwinds the double helix, especially in the case of intrastrand cross-links to G-rich sequences such as GG and AG [2, 3]. Cisplatin also inflicts mitochondrial damage, induces cell cycle arrest in the G2 phase, reduces ATPase activity, alters cellular transport system, eventually leading to apoptotic and/or necrotic cell death.

Cisplatin is the single most active antitumor agent against testicular, bladder, ovarian, lung, head and neck tumors (table 1). The use of cisplatin in combination with drugs such as bleomycin, vinblastine, cyclophosphamide, fluorouracil and doxorubicin has resulted not only in higher effectiveness in treating various tumors, but has also increased the risk of secondary morbidity. Although cisplatin was first synthesized in 1845, the side effects associated with cisplatin treatment was not adequately described until 1965. Cisplatin entered into clinical trials in and around 1971. Despite its effectiveness as an antitumor drug, various side effects (table 2), especially nephrotoxicity, has restricted its clinical use. The nephrotoxic effect of cisplatin is dose limiting [4, 5], and is manifested by a decrease in creatinine clearance and electrolyte imbalances, particularly hypomagnesemia, mainly due to the acute cytotoxic

**Table 2.** Partial list of side effects of cisplatin

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Acute encephalopathy
Anaphylactic reactions
Elevated liver function tests
Hair loss
Hearing loss
Hemolytic anemia
Infertility
Mucositis
Myelosuppression
Nausea and vomiting
Optic neuropathy
Peripheral neuropathy
Raynaud's syndrome
Retinopathy
Tinnitus
Nephrotoxicity

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effect of cisplatin on proximal and distal tubules, and on loop of Henle [6]. Severe magnesium deficiency following cisplatin treatment could result in seizures [7]. Cisplatin-induced excessive urinary loss of magnesium and potassium [8] could be partly restored by supplementation [9, 10]. In addition, both human and experimental studies have shown that the use of diuretics and hydration can substantially reduce cisplatin-associated nephrotoxicity [11, 12].

A detailed and comprehensive review of all aspects of cisplatin-associated toxicity is beyond the scope of this article, which will thus be restricted to various pathological events of cisplatin-associated nephrotoxicity.

### **Cisplatin and Nephrotoxicity**

Cisplatin-induced nephrotoxicity is a complex process that comprises of acute cytotoxic effects on tubular epithelial cells, resulting in loss of tubular epithelial cells by necrosis and apoptosis, followed by inflammatory cell infiltration and fibroproliferative changes [13]. From in vivo experimental studies, the progression of cisplatin-induced renal damages can be tentatively divided into three main events, which at times may overlap: initial cytotoxic, inflammatory and fibroproliferative events.

#### *Initial Cytotoxic Events*

It has been convincingly demonstrated that renal tubular dysfunction is the immediate effect of cisplatin treatment. Higher doses of cisplatin induce

necrosis of tubular epithelial cells, while lower doses remove tubular epithelial cells via apoptosis [14–16]. Cisplatin exerts its cytotoxic effects partly by inhibiting protein synthesis of tubular epithelial cells. Besides, cisplatin disrupts the cellular oxidant defense system (i.e., glutathione, GSH), leading to lipid peroxidation and DNA damage. Cisplatin-associated cytotoxicity and generation of reactive oxygen species (ROS) could be counteracted by using antioxidants such as  $\alpha$ -tocopherol, vitamin C and *N*-acetylcysteine [17, 18]. Nephrotoxicity induced by high-doses of cisplatin therapy could be altered by GSH administration [19–22]. GSH treatment could also protect nerve injury following cisplatin therapy, without reducing its antitumor activities [23–25]. A protective role of metallothionein, a scavenger of hydroxyl radicals, against a number of oxidative stress-associated xenobiotics, including cisplatin, has been reported by Bauman et al. [26]. Renal proximal tubular epithelial cells (LLC-PK1), stably transfected with human HSP72 gene, have shown to be resistant to both hydrogen peroxide and cisplatin-induced cellular damage, implicating a protective role of heat shock protein 72 (HSP72) against oxidative injury and cisplatin toxicity [27].

Cisplatin could also activate various proapoptotic molecules including caspase-3 and -9, Bax and Fas system [14, 28, 29]. In vitro studies have shown that cisplatin-induced apoptosis in LLC-PK1 is mediated through activation of mitochondrial signaling pathways, possibly by activating Bax-induced mitochondrial permeability, with release of cytochrome c and activation of caspase-9. A role of caspase-3 has also been reported in cisplatin-induced apoptosis in LLC-PK1 cells, and shown to be prevented by bcl-2 [30]. Moreover, a relationship between loss of cytoskeletal F-actin stress fibers and cisplatin-induced apoptosis has been shown in renal epithelial cells (within 4–6 h), and prevention of F-actin damage by phalloidin has shown to prevent nuclear fragmentation of these cells [31]. van de Water et al. [32] reported that decreased phosphorylation of focal adhesion kinase was related to loss of focal adhesions and F-actin stress fibers, leading to the onset of apoptosis in renal tubular epithelial cells caused by nephrotoxicants. In addition, involvement of Fas/Fas ligand system has been demonstrated in cisplatin-induced apoptosis in various cells lines [33–37]. Cisplatin-induced apoptosis in human proximal tubular epithelial cells was associated with an increased expression of Fas and its ligand [37]. Similar Fas-mediated cisplatin-induced apoptosis has been reported in neuroblastoma [36], leukemia [35] and hepatoma cells [34] and thymocytes [33]; in contrast a Fas-independent cisplatin-induced apoptosis has also been reported in various tumors cell lines [38, 39] including lung cancer cells. It appears likely that cisplatin-induced apoptosis does not always take a uniform pathway, and there might be a cell-specific mode of apoptosis. Early cytotoxic events following cisplatin treatment are usually associated with inflammatory changes in the kidneys.

### *Inflammatory Events*

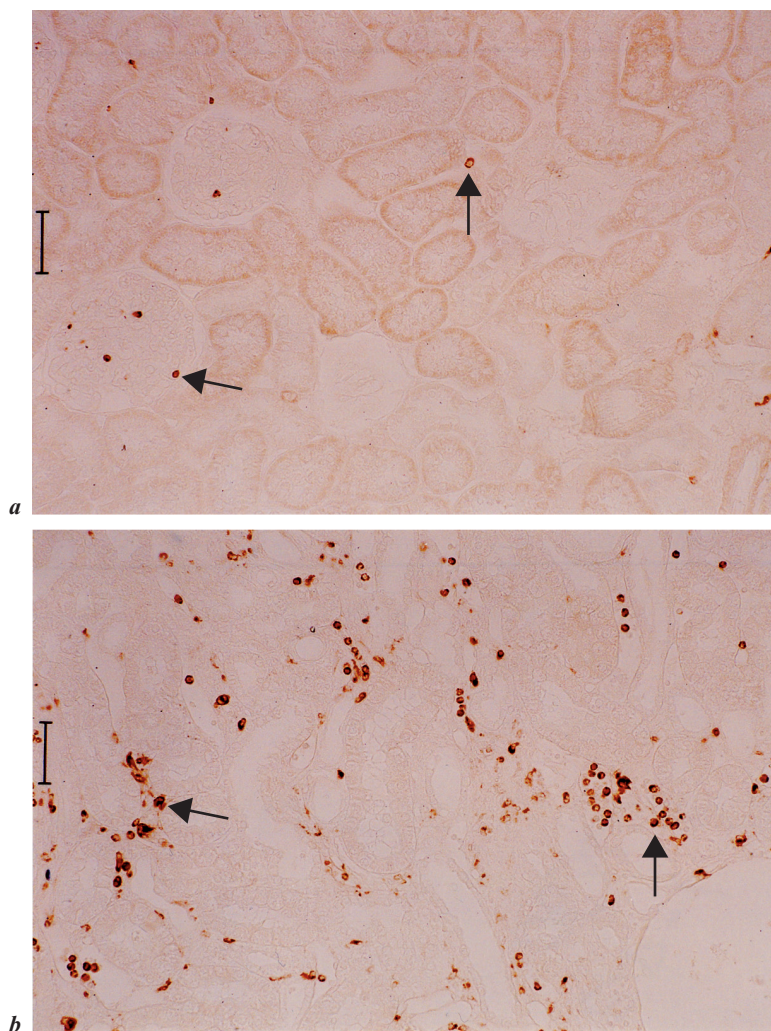
Detailed inflammatory phenotypes of infiltrating cells in kidney of cisplatin-treated patients are not well studied, but data from animal experiments have shown that by day 7, a single dose of cisplatin injection (6 mg/kg body weight) to rats lead to the accumulation of a maximum number of ED-1-positive macrophages in the cortico-medullary junction of the kidneys (fig. 1). The number of accumulated macrophages declined on day 14 and 28 [40–42]. Macrophages, through generation of ROS, could intensify cytotoxic effects encountered following cisplatin treatment.

It is well accepted that cytokines and chemokines play a major role in the inflammatory events of various human and experimental diseases. Cisplatin has been reported to induce the expression of inflammatory cytokines, such as interleukin (IL)-1 and IL-6 by endothelial cells isolated from a human umbilical vein [43]. Increased renal expression of tumor necrosis factor- $\alpha$ , transforming growth factor (TGF)- $\beta$ , RANTES, macrophage inflammatory protein-2, macrophage chemoattractant protein-1, thymus-derived chemotactic agent 3, IL-1 $\beta$  and intercellular adhesion molecule-1 has been detected in kidneys of cisplatin-treated animals [44]. Recently, salicylate has been shown to reduce experimental cisplatin nephrotoxicity, by inhibition of tumor necrosis factor- $\alpha$  production through stabilization of I  $\kappa$  B [45]. Moreover, increased interstitial expression of osteopontin has been detected in the kidneys of cisplatin-treated rats [46]. It is likely that tubular epithelial cell-derived chemokines and ROS following cisplatin treatment serve to recruit inflammatory cells, which can contribute to the development of subsequent fibroproliferative lesions by releasing mitogenic and fibrogenic factors, which then act on matrix-producing cells to regulate abnormal matrix remodeling.

### *Fibroproliferative Events*

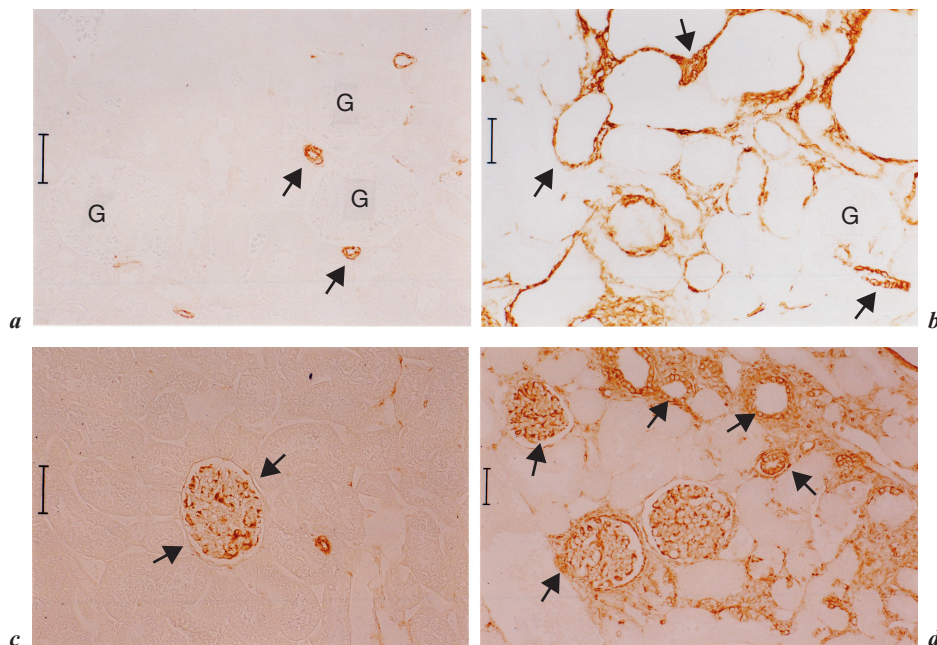
Development of irreversible tubulointerstitial fibrosis is a relatively late change found in the kidneys of cisplatin-treated experimental animals. Excessive production of matrix proteins by the activated and phenotypically altered resident cells gradually help in the development of tubulointerstitial fibrosis. An increased expression and deposition of collagens (types I, III and IV) were detected in cisplatin-induced tubulointerstitial fibrosis in rats [47], a pattern that is similar to other experimental models of tubulointerstitial fibrosis [48–51].

Fibrogenic factors, released by the activated and phenotypically altered resident cells (fig. 2) and infiltrating inflammatory cells, such as TGF- $\beta$ 1 and HSP47, have the potential to mediate both human and experimental fibrotic diseases by regulating increased production of collagens, and thereby matrix remodeling [51–54]. TGF- $\beta$ 1 affects formation of connective tissue by



**Fig. 1.** Infiltration of ED-1-positive macrophages (arrows) in control (*a*) and cisplatin-treated rat kidneys (*b*). Note a significantly increased accumulation of macrophages (arrows) in cisplatin-treated rat kidney (*b*).

stimulating the transcription of genes encoding extra cellular matrix proteins. Studies have convincingly demonstrated that blocking TGF- $\beta$ 1 results in the suppression of collagen production and subsequent modulation of fibrotic processes [55, 56]. A fibrogenic role for TGF- $\beta$ 1 has been reported in kidneys of patients with various renal diseases [54, 55, 57]. In the kidneys of cisplatin-treated rats, an increased expression of TGF- $\beta$ 1 has been detected in tubular



**Fig. 2.** Immunostaining of  $\alpha$ -smooth muscle actin in a control rat kidney (*a*), showing positive staining mainly in the vessel walls (arrows); increased interstitial expression of  $\alpha$ -smooth muscle actin (arrowheads) is noted in cisplatin-treated rat kidney (*b*), suggesting phenotypically altered myofibroblast proliferation following cisplatin treatment. No significant expression of  $\alpha$ -smooth muscle actin was detected in the glomeruli (denoted as G) in both control and kidneys of cisplatin-treated rat. For vimentin, only intraglomerular staining (arrows) is noted in the control rat kidney (*c*). Note no staining for vimentin in the tubular epithelial cells in the control rat kidney. Strong positive staining for vimentin is noted in the tubular epithelial cells (arrowheads) and interstitial cells in cisplatin-treated rat kidney (*d*), suggesting phenotypically altered tubular epithelial cells following cisplatin treatment.

epithelial cells and interstitial cells, by in situ hybridization [58]. Further studies are needed to determine the effects of increased expression of TGF- $\beta$ 1 in cisplatin nephritis, and the role of TGF- $\beta$ 1-induced molecules, including connective tissue growth factor, in such fibroproliferative lesions [59–61]. In addition, c-myc, ets-1, platelet-derived growth factor, ILs, interferon- $\gamma$ , tumor necrosis factor, epidermal growth factor, insulin-like growth factor and its binding proteins, angiotensin II and tissue transglutaminase, have shown to play roles in the development of fibroproliferative lesions in various human and experimental renal diseases. Interestingly, by microarray analysis, a number of these above-mentioned molecules were detected in the kidneys of cisplatin-treated rats [62].

HSP47, a collagen-specific molecular chaperone, is involved in the biosynthesis and secretion of procollagens [63]. HSP47 has shown to play important role in the development of fibroproliferative changes by post-transcriptionally regulating increased production of collagens. For instance, upregulation in the expression of HSP47 with increased interstitial accumulation of collagens (types I and III) has been reported in various human and experimental fibrotic renal diseases [53, 64, 65]. Similar upregulation of HSP47, in association with increased accumulation of type I and III collagens, was also detected in kidneys of cisplatin-treated rats [47]. Phenotypically altered tubular epithelial cells, interstitial fibroblasts and myofibroblasts were HSP47-expressing cells in kidneys of cisplatin-treated rats [47]. Although further studies are warranted, at this stage, HSP47 appears to play a role in the development of fibroproliferative lesions in the kidneys following cisplatin treatment. In addition to HSP47, induction of several other HSPs (HSP-70, -90) has been reported during early stages of cisplatin nephropathy [66].

Production of extracellular matrix is mainly achieved through the synthesis of collagens, whereas resorption of the extracellular matrix is mediated predominantly by the matrix metalloproteinases (MMPs). A delicate balance between matrix synthesis and its degrading enzymes (MMPs) is essential for maintaining normal structural stability and integrity of tissues and organs. An imbalance in the production and utilization of matrix proteins lead to pathological matrix remodeling. In the kidneys of cisplatin-treated rats, the expression of MMP-1 has shown to increase in early stages (on day 3) of cisplatin nephropathy, while the expression decreased in later stages (on day 14). Decreased renal expression of MMP-1 has been shown to be associated with increased interstitial accumulation of type III collagen in kidneys of cisplatin-treated rats [67], suggesting a pathological role of MMPs in cisplatin-nephropathy.

### **Modulation of Cisplatin-Induced Nephrotoxicity**

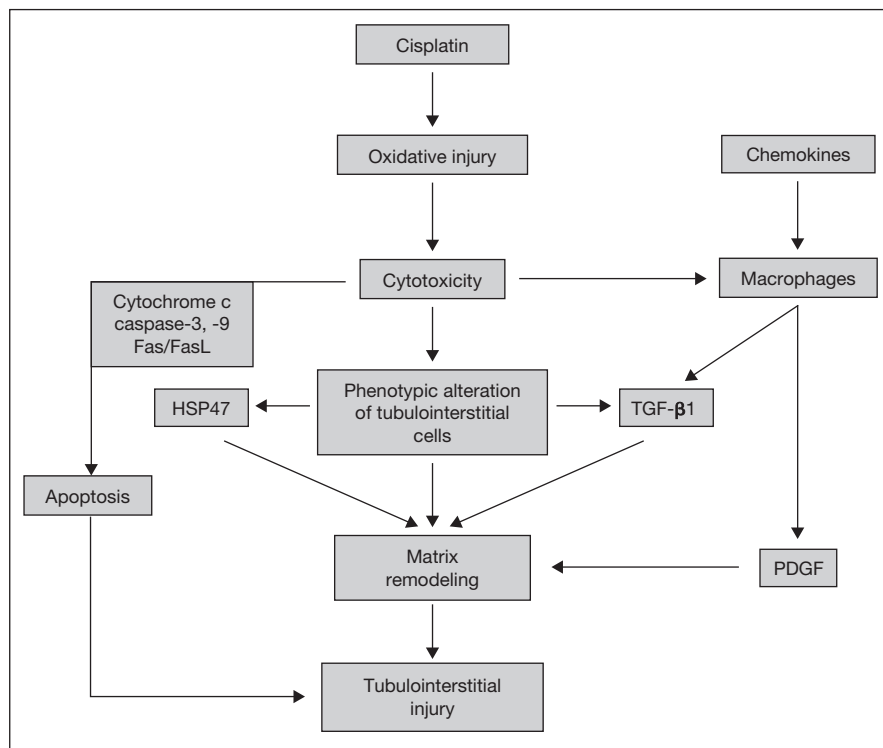
The beneficial antineoplastic use of cisplatin is often limited because of its significant side effects, including nephrotoxicity. Following standard-dose regimens, one third of patients usually develop varying degrees of cisplatin-related side effects. Numerous human and experimental studies have been performed to understand the mechanism of cisplatin-associated nephrotoxicity, and thereby to minimize its side effects. Several strategies have been explored to reduce the side effects of cisplatin therapy, including the use of less intensive treatment, replacement of the nephro- and neurotoxic cisplatin by its less toxic analog carboplatin. Carboplatin generates a reactive species much more slowly than with cisplatin. Therefore its pharmacokinetic and toxicological



characteristics are different. Moreover, plasma half-life of carboplatin is several-fold longer than that of cisplatin. Needless to mention that carboplatin also exerts unwarranted side effects that include fatigue, bone marrow dysfunction and loss of fertility. Aggressive hydration with saline, often with the addition of mannitol, has been used to reduce cisplatin-induced nephrotoxicity. Two liters of 5% dextrose in 0.5 *N* saline over 12–24 h before treatment and at least 24 h of intravenous fluid afterward is helpful in minimizing the kidney damage after cisplatin treatment.

Amifostine (Ethyol) is an organic thiophosphate compound with a cytoprotective potential. The active free thiol metabolite can reduce the toxic effects of cisplatin on the kidney, possibly by binding to free radicals generated in the tissues. Patients treated with amifostine prior to cisplatin therapy were reported to have less renal damage compared with patients treated with cisplatin alone [68–70]. In experimental models, preadministration of a zinc-histidine complex has been reported to reduce cisplatin-induced renal damage, possibly by preventing peroxidative damage [71]. Recently heme oxygenase-1 (HO-1), a 32-kDa microsomal enzyme, has been shown to attenuate cisplatin-induced apoptosis and necrosis. It has been shown that compared to wild-type mice (HO-1<sup>+/+</sup>), cisplatin-treatment intensified renal injury in homozygous mice with a targeted deletion of the HO-1 gene (HO-1<sup>-/-</sup>) [72]. Studies have also shown that the upregulation of p21, a cyclin-dependent kinase inhibitor, attenuated cisplatin-induced renal dysfunction, apoptotic cell death and tubular damage [73]. A protective role of p21 has also been shown in p21 knockout mice treated with cisplatin [74].

In vitro treatment of renal epithelial cells (mIMCD-3) with cisplatin could induce apoptosis, while constitutive expression of hepatocyte growth factor by transfection in mIMCD-3 cells developed resistance to cisplatin-induced apoptotic death, implicating that hepatocyte growth factor may ameliorate cisplatin-associated renal injury, by protecting renal epithelial cells from undergoing apoptosis [75]. Cisplatin-associated nephrotoxicity has been reported to be modified by taurine treatment in rats. Compared to cisplatin-treated rats, taurine-treated rats showed relatively less renal damage, as determined by histo-morphometric analysis. Taurine-treatment resulted in less macrophage accumulation and delayed interstitial fibrotic changes in cisplatin-treated rat kidneys [76, 77]. Recently, ebselen has shown to be nephroprotective in cisplatin-treated rats, possibly exerting its beneficial effects by modulating the antioxidant system [78, 79]. Similarly, treatment of myeloma cells with N-acetylcysteine completely blocked cisplatin-associated intracellular GSH oxidation, ROS generation, poly(ADP-ribose) polymerase cleavage and apoptosis [80]. Use of a novel free radical scavenger, 3-methyl-1-phenyl-pyrazolin-5-one (MCI-186; edarabone) has also been shown to protect the kidneys from



**Fig. 3.** Schematic diagram showing involvement of various molecules involved in initiation and progression of cisplatin-induced nephrotoxicity. TGF- $\beta$ 1 = Transforming growth factor; PDGF = platelet-derived growth factor; HSP47 = heat shock protein 47.

developing acute renal failure following cisplatin treatment [81]; edarabone, a lipophilic compound, has been shown to trap both hydroxyl radicals and prevent iron-induced peroxidative injuries [82]. These studies suggest a beneficial role in the use of a free radical scavenger in modulating cisplatin-associated nephrotoxicity.

### Conclusion

Despite prophylactic intensive hydration and forced diuresis, irreversible renal damage occurs in about one third of cisplatin-treated patients. Cisplatin-induced renal damage is usually associated with acute stress-related injuries, focal necrosis and apoptosis of the tubular epithelial cells and dilatation of tubules with cast formation. Inflammatory events initiated due to cytotoxic

stress responses of cisplatin facilitate activation of resident cells to release of profibrogenic factors, which induces excessive production of matrix proteins, resulting in irreversible tubulointerstitial injuries (fig. 3). Further studies characterizing the molecules involved in acute stress responses following cisplatin treatment, and determining their molecular interactions in various stages of nephrotoxicity, would help in developing strategies to make a focused approach to minimize cisplatin-associated nephrotoxicity, without reducing or interfering with its antitumor effects. At this stage, modulating oxidative stress following cisplatin treatment appears to be a promising option to reduce its side effects, including nephrotoxicity.

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