

Review

## Comparative Nephrotoxicity of Cisplatin and Nedaplatin: Mechanisms and Histopathological Characteristics

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**Abstract:** The antineoplastic platinum complexes cisplatin and its analogues are widely used in the chemotherapy of a variety of human malignancies, and are especially active against several types of cancers. Nedaplatin is a second-generation platinum complex with reduced nephrotoxicity. However, their use commonly causes nephrotoxicity due to a lack of tumor tissue selectivity. Several recent studies have provided significant insights into the molecular and histopathological events associated with nedaplatin nephrotoxicity. In this review, we summarize findings concerning the renal histopathology and molecular pathogenesis induced by antineoplastic platinum complexes, with a particular focus on the comparative nephrotoxicity of cisplatin and nedaplatin in rats. (DOI: 10.1293/tox.24.87; J Toxicol Pathol 2011; 24: 87–94)

**Key words:** cisplatin, kidney, nedaplatin, nephrotoxicity, renal papilla, toxicogenomics

### Introduction

Antineoplastic platinum complexes are widely used to treat several human malignancies. Cisplatin, a first-generation platinum complex, has been successfully used for treatment of several types of cancer, including testicular, head and neck, ovarian, cervical and nonsmall cell lung carcinoma<sup>1–4</sup>. Although the precise mechanism has not been fully elucidated, the possible mechanisms involved in the anticancer activity of cisplatin are becoming clearer. Cisplatin directly binds to the DNA of tumor cells, forming a cross-link that leads to the arrest of DNA synthesis and replication. In the case of rapidly dividing cells such as cancers, cisplatin can also induce DNA damage, which ultimately leads to irreversible cellular injury and death.

Unfortunately, cisplatin treatment causes several types of side effects due to the drug's lack of target selectivity. The major limiting factors in the use of cisplatin are neurotoxicity, ototoxicity, nausea, vomiting and nephrotoxicity. Among these factors, the prevalence of nephrotoxicity is extremely high, occurring in about one-third of patients undergoing cisplatin treatment<sup>5–7</sup>. Nephrotoxicity is therefore regarded as the most common and serious side effect of cisplatin che-

motherapy<sup>8–10</sup>.

Over the last two decades, hundreds of platinum complexes have been synthesized and tested to lessen the adverse effects as well as to improve the effectiveness of these antineoplastic agents<sup>11,12</sup>. Dose-limiting nephrotoxicity has been a critical concern in the selection of new drug candidates during the early stages of drug development. Several novel cisplatin analogues have been identified that exhibit less severe side effects without sacrificing their beneficial effects. Nedaplatin is one of the second-generation platinum complexes with reduced nephrotoxicity and may serve as a substitute for cisplatin or even surpass cisplatin for use in combination with other drugs. Nedaplatin's spectrum of activity largely overlaps that of cisplatin, as the drug is active against various solid tumors, including lung cancer<sup>13</sup>, esophageal cancer<sup>14</sup>, head and neck cancer<sup>15</sup>, testicular cancer<sup>16</sup> and ovarian cancer<sup>17</sup>. However, nedaplatin also causes nephrotoxicity at doses that are therapeutic in humans. In this review, we summarize recent findings concerning the renal histopathology and molecular pathogenesis induced by antineoplastic platinum complexes, with a particular focus on the comparative nephrotoxicity of cisplatin and nedaplatin in rats.

### Renal Histopathology

#### *Comparative nephrotoxicity of cisplatin and nedaplatin*

Toxicity target and histopathological characteristics in cisplatin and nedaplatin nephrotoxicity are summarized in Table 1. Renal tubular cell death is a common histopatho-

**Table 1.** Toxicity Target and Histopathological Characteristics in Cisplatin and Nedaplatin-induced Nephrotoxicity in Rats

Compounds	Toxicity target in the kidney	Histopathology
Cisplatin	Proximal tubule, straight (S3 segment) in the outer medullary stripe	Necrosis (degeneration), apoptosis and regeneration of the renal proximal tubule; tubular dilatation; inflammatory cells infiltration; fibrosis (chronic)
Nedaplatin	Renal papilla (collecting duct, epithelium covering the papilla)	Papillary necrosis (severe case only); hyaline droplet (enlarged lysosomes), necrosis (degeneration), apoptosis and regenerative hyperplasia of the collecting duct epithelium and the epithelium covering the renal papilla; tubular dilatation; with slightly affected proximal and distal tubules in the renal cortex

logical feature associated with nephrotoxicity induced by antineoplastic platinum complexes. In *in vivo* animal models, cisplatin primarily damages the proximal tubule, specifically the S3 segment of the proximal tubule in the outer medullary stripe<sup>18–20</sup>. The histopathological characteristics of cisplatin nephrotoxicity in rats are massive necrosis and subsequent regeneration of renal proximal tubular cells<sup>21–23</sup>. In contrast, renal papillary injury is reported to be minor, and the glomerulus undergoes no obvious morphologic changes<sup>24,25</sup>. Long-term cisplatin treatment causes cyst formation and interstitial fibrosis in the kidney<sup>22</sup>.

In contrast to cisplatin nephrotoxicity, nedaplatin primarily affects the renal papilla in rats<sup>23</sup>. Papillary injury induced by nedaplatin is morphologically characterized by hyaline droplet changes, apoptosis or regenerative hyperplasia of the collecting duct epithelium and the epithelium covering the renal papilla. These adverse changes in the renal papilla tend to be greater toward the tip. In severe cases, papillary necrosis also occurs. Ultrastructural features in combination with a positive reaction to acid phosphatase confirm that the hyaline droplets associated with nedaplatin nephrotoxicity are hyperplastic lysosomes. In the cortex, only slight necrotic and degenerative changes are observed, such as slight focal tubular necrosis, regeneration and dilatations lined with a flattened epithelium in the proximal and distal tubule. The glomerulus shows no obvious toxic changes.

#### *Time course of the changes of nedaplatin nephrotoxicity*

We also characterized the temporal changes in nedaplatin nephrotoxicity in rats<sup>26</sup>. The early stage of nedaplatin-induced nephrotoxicity in rats is characterized by apoptosis, with shrunken cytoplasm and pyknotic/fragmented nuclei in the collecting duct epithelial cells and proximal and distal tubular cells. Four days after nedaplatin dosing, a small number of cortical tubules exhibit focal necrosis, and are filled with debris derived from necrotic and/or apoptotic cells. The cortical tubule also exhibits a degenerative appearance and is characterized by loss of brush borders and abnormally large vacuoles in the cytoplasm. In the period 4 to 7 days following nedaplatin treatment, cystic dilatation and regeneration of the renal tubules occur, with atypical nuclei in the renal cortex. In the collecting duct epithelium,

morphological regenerative changes reflecting the tubular injury occur and are characterized by protrusion into the collecting duct lumen of enlarged cells with atypical nuclei. Characteristic papillary necrosis is also observed in the tip of renal papilla 4 or more days after a single nedaplatin dose<sup>24,26,27</sup>. In the later stage of nedaplatin-induced nephrotoxicity, subsequent cystic dilatation and regeneration occur in the affected tubules, but incomplete tissue repair is still observed 2 weeks after treatment.

### **Pathogenesis of Nephrotoxicity**

#### *Renal uptake and accumulation*

To exert cytotoxic activity, the platinum complex must enter a cell. Cisplatin is preferentially taken up by the highly susceptible proximal tubular cells within the S3 segment, the principal site of acute injury in the kidney<sup>18–20</sup>. Safirstein *et al.*<sup>28</sup> demonstrated renal preference in the accumulation of cisplatin; in renal tissue slices, they found cisplatin at concentrations up to five-fold above the concentration in the medium. Safirstein *et al.* also demonstrated that the kidney accumulates platinum in part by transport or specific binding to components of the kidney transport system and then biotransforms the platinum intracellularly. Preferential injury to the proximal tubule could therefore be explained by the propensity of cisplatin to accumulate in this tissue.

Although the precise mechanisms involved in the intracellular transport systems of platinum complexes are unclear, several reports have discussed the cellular uptake of platinum complexes under both *in vitro* and *in vivo* conditions. In the case of cancer cells, about half of the cisplatin taken up is due to passive diffusion through the plasma membrane, and uptake of the remaining half is mediated by several factors, including transporters<sup>29</sup>. A high-affinity copper transporter, Ctr1, is believed to play a role in cisplatin uptake in cancer cells. Recent works clearly indicate that Ctr1 protein localizes mainly on the basolateral side of tubular cells in the kidney and contributes to cisplatin uptake by these cells<sup>30</sup>. Thus, renal Ctr1 expression might be responsible for cisplatin-induced tubular toxicity. In addition, several reports suggest that organic cation transporters (Oct), specifically Oct2, are involved in the uptake of cisplatin in renal tubular cells. Ciarimboli *et al.*<sup>31</sup> confirmed that

human Oct2 is responsible for basolateral uptake of cisplatin in the kidney. Yonezawa *et al.*<sup>32</sup> determined that in HEK293 cells stably expressing rat Oct2, renal Oct2 expression is the major determinant of cisplatin-induced tubular toxicity. Filipinski *et al.*<sup>33</sup> provided evidence that Oct1/Oct2-deficient mice are protected from renal tubular damage caused by cisplatin. Subsequently, they found that a nonsynonymous single-nucleotide polymorphism in the Oct2 gene is associated with reduced cisplatin-induced nephrotoxicity in humans.

Information regarding the mechanisms involved in the cellular uptake of nedaplatin is relatively limited. However, there is clear evidence that in both humans and rats, nedaplatin, which is less nephrotoxic than cisplatin, does not interact with Oct2 or the apical multidrug and toxin extrusion transporter (MATE) family of proteins<sup>34,35</sup>. The reduced nephrotoxicity of nedaplatin might therefore be associated with reduced accumulation in the proximal tubules due to its lower affinity for transporters, including Oct2 and the MATE family. Tanaka *et al.*<sup>36</sup> reported that 10 min after a single intravenous dose of radiolabeled nedaplatin, the highest radioactivity is found in the kidney, particularly in the cortex and papilla. After 1 h, the highest radioactivity is observed in the papilla. Differences in the primary site of injury in the kidney may therefore be partially attributed to differences in the renal kinetics of cisplatin and nedaplatin. In addition, histopathological differences may be due to differences in the transporter systems involved, but additional studies are needed to resolve this question.

#### *Molecular pathogenesis of cisplatin nephrotoxicity*

Oxidative stress is one of the main mechanisms of cisplatin nephrotoxicity<sup>37</sup>. Various reactive oxygen species (ROS) are produced by cisplatin treatment in cultured renal tubular cells, kidney slices and *in vivo* in the kidney. Several possible mechanisms have been proposed to account for ROS generation under the pathological conditions of cisplatin nephrotoxicity. After being incorporated into a cell, cisplatin is transformed to a highly reactive form, which then rapidly reacts with antioxidants including glutathione. However, under conditions in which there is excessive generation of the reactive form in a cell, glutathione and related antioxidants are depressed, leading to a change in the redox status and eventually resulting in the accumulation of endogenous ROS. ROS in turn causes oxidative damage within the cells<sup>38</sup>. In addition, mitochondrial dysfunction might be related to increased ROS production in cisplatin nephrotoxicity due to disruption of the mitochondrial antioxidant defense system<sup>39</sup>. Although the molecular targets of ROS generated by cisplatin treatment are still largely unknown, ROS may react with multiple molecules in the cell, including mitochondrial lipids and proteins, ultimately leading to cellular injury.

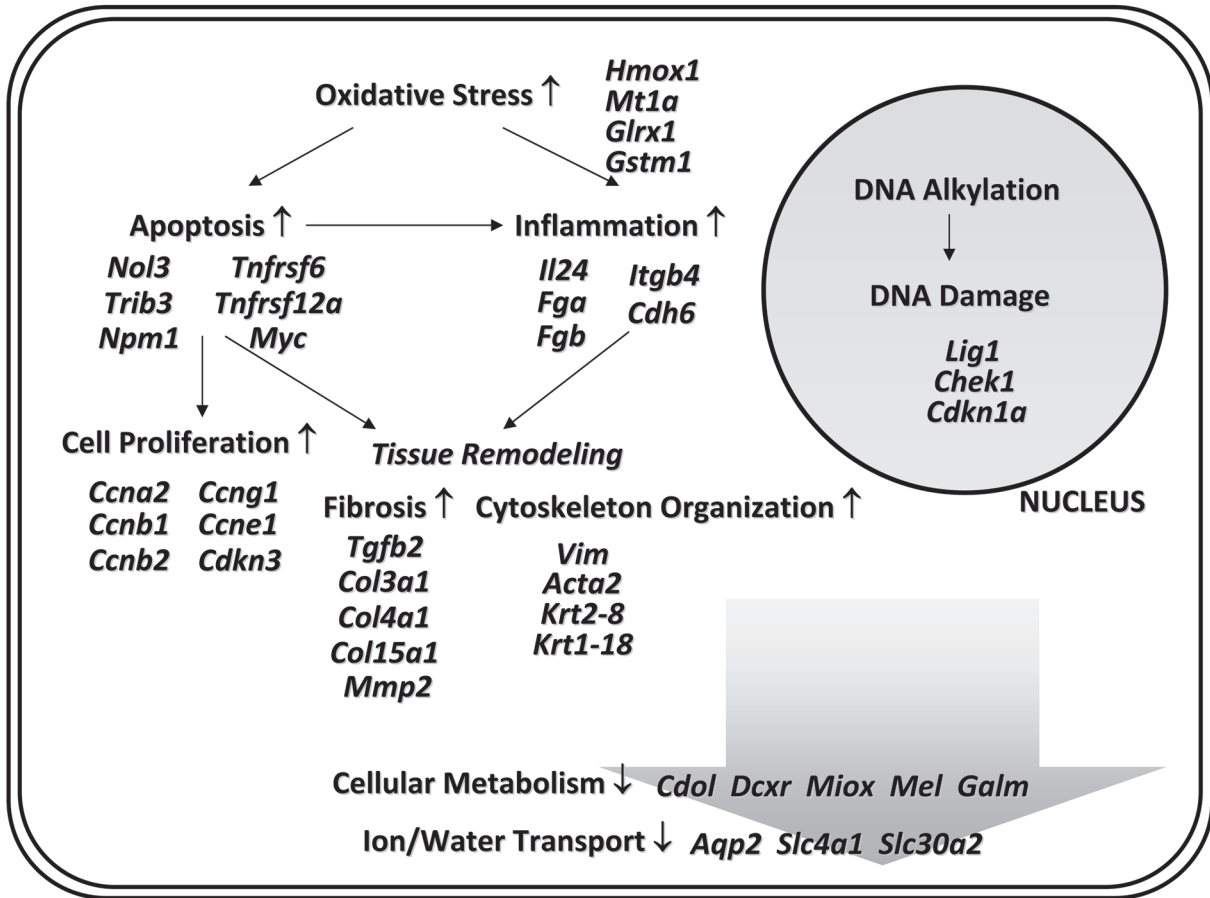
At the molecular level, several genes are known to be deregulated in the kidney by cisplatin treatment. Comprehensive gene expression profiling revealed that oxidative stress response genes are deregulated following 7-day cisplatin treatment in rats. This includes upregulation of the

gene encoding metallothionein 1 and downregulation of genes encoding catalase, superoxide dismutase and  $\gamma$ -GT<sup>40</sup>. Aleksunes *et al.*<sup>41</sup> demonstrated the induction of cytoprotective genes/proteins following cisplatin treatment, including NAD(P)H dehydrogenase, quinone 1, heme oxygenase 1 (Hmox1) and glutamate cysteine ligase catalytic subunit, all of which are known to be induced by activation of the transcription factor Nrf2 (nuclear factor E2-related factor 2). These changes in gene expression could be responsible for downstream cell death associated with oxidative stress-mediated tubular injury in cisplatin nephrotoxicity. There is also some evidence suggesting that cisplatin treatment induces glutathione depletion in the kidney<sup>42</sup> and that glutathione supplementation attenuates cisplatin nephrotoxicity<sup>43</sup>. Taken together, the available evidence indicates that oxidative stress plays an important role in the mechanism of cisplatin nephrotoxicity.

Excessive oxidative stress leads to the death of renal tubular cells as a result of cisplatin-induced cellular injury, which leads to tubular cell necrosis and apoptosis. Several apoptotic pathways have been implicated in this process, including the extrinsic pathway mediated by death receptors, the endoplasmic reticulum-stress pathway and the mitochondrial-associated intrinsic apoptotic pathway<sup>12,44,45</sup>. In addition to the involvement of these apoptotic pathways, p21 is also known to play an important role in directing the switching of signals in renal tubular cells between survival cell death<sup>46,47</sup>. Both necrosis and apoptosis are reported to be induced in renal tubular cells *in vivo*<sup>48,49</sup>. This difference of phenotype in cisplatin-induced cellular injury can be explained by evidence demonstrating that exposure to high concentrations of cisplatin lead to necrotic cell death, while exposure to much lower concentrations result in apoptosis<sup>50</sup>. Furthermore, it has also been determined that ROS play a role in mediating apoptosis but not necrosis following cisplatin exposure<sup>50</sup>. Thus, the mechanism of cisplatin-induced renal tubular necrosis is thought to be concentration-dependent.

#### *Molecular pathogenesis of nedaplatin nephrotoxicity*

Application of toxicogenomics is a promising strategy to determine the molecular mechanisms underlying chemical toxicity and to aid in the early detection of toxic effects<sup>51–54</sup>. Recently, our laboratory conducted a toxicogenomic study to broaden understanding of the cellular and molecular mechanisms involved in nedaplatin nephrotoxicity<sup>50</sup>. Using DNA microarrays, gene expression profiles were analyzed in two renal regions, the cortex and papilla, following administration of a single dose of nedaplatin in rats. We found striking similarities in the gene expression profiles between the renal cortex and papilla. More specifically, we found that several genes belonging to various functional categories are deregulated in both renal regions in accordance with histopathological changes associated with nedaplatin nephrotoxicity (Fig. 1). Upregulated genes include those involved in apoptosis, cell cycle regulation, DNA metabolism, cell migration/adhesion, cytoskeleton organization and oxidative



**Fig. 1.** Putative cellular pathways of nedaplatin nephrotoxicity. This schematic pathway was assembled using gene expression data obtained from nedaplatin-treated rat kidneys 6 days after a single dosing. Upregulation and downregulation are represented by ↑ or ↓, respectively.

stress, while genes reflecting renal malfunction, including calcium homeostasis, are downregulated. One of the major histopathological changes associated with nedaplatin nephrotoxicity is apoptosis in renal tubules. Upregulation of myelocytomatosis viral oncogene homolog, death-associated protein, tribbles homolog 3 and TNF receptor superfamily member 6 in nedaplatin nephrotoxicity is thought to be related to apoptotic cell death. In addition, we also observed that the expression of various oxidative stress responsive genes, including *Hmox1*, metallothionein 1a, glutaredoxin 1 and glutathione S-transferase mu 1, is enhanced, as is the case in cisplatin nephrotoxicity. The *Hmox1* gene encodes the rate-limiting enzyme in the degradation of heme<sup>55</sup>, and protects against oxidative stress<sup>56,57</sup>. Metallothioneins play a role in the detoxification of toxic metals and in protection against oxidative stress<sup>58–60</sup>. Glutaredoxins are thiol oxidoreductases that use reduced glutathione as a hydrogen donor to reduce protein disulfides or glutathione–protein-mixed disulfides in a coupled system with glutathione reductase and NADPH<sup>61,62</sup>. Glutathione S-transferase, a member of the phase II group of xenobiotic metabolizing enzymes, is known to conjugate reactive compounds to glutathione prior to their excretion from the body<sup>63</sup>, and also acts as a gluta-

thione peroxidase, converting lipid peroxides to the hydroxyl form and oxidizing reduced glutathione<sup>63</sup>. On the basis of these observations, we concluded that nedaplatin treatment affects both the renal papilla and cortex through oxidative stress-related mechanisms.

Our research also provided novel evidence indicating that cytokeratin subtypes 14 and 19 are overexpressed in the papilla following nedaplatin treatment<sup>50</sup>. Distinct positive expression of cytokeratins 14 and 19 was specifically localized to the epithelium covering the papilla and/or the collecting duct epithelium. Cytokeratins, which are a diverse group of intermediate filament proteins, are known to play a critical role in differentiation and tissue specialization and function in the maintenance of epithelial cell structural integrity. The wide acceptance of cytokeratins as excellent epithelial cell markers is largely related to the differentiation-specific expression patterns shown by individual isoforms<sup>64–66</sup>. Cytokeratin 14 is expressed in the basal and first suprabasal layers of various stratified squamous epithelia, but not in simple epithelial cells<sup>67</sup>. Cytokeratin 19 is a major component of simple epithelia in a wide range of epithelial tissues<sup>64</sup>. In nedaplatin-induced renal papillary toxicity, increased expression of cytokeratins 14 and 19 might reflect

abnormal squamous differentiation of the epithelium covering the renal papilla and regeneration related to the repair process of the injured epithelial cells in the collecting duct.

### Amelioration of Nedaplatin Nephrotoxicity

As described above, nedaplatin has the potential to affect the entire nephron, with characteristic papillary injury occurring where the drug concentration reaches maximum levels<sup>46,48</sup>. Hydration is thought of as a promising approach for ameliorating cisplatin nephrotoxicity because it promotes excretion of the drug into the urine and dilutes the drug concentration in the urine by causing polyuria<sup>7,11,68</sup>. We evaluated whether hydration before dosing does in fact ameliorate nedaplatin nephrotoxicity. We found that hydration dramatically reduces nedaplatin nephrotoxicity, but has no clear effect on myelotoxicity. Measurement of urinary platinum excretion revealed that the total amount of platinum excreted is significantly higher in hydrated rats than in nonhydrated rats. In terms of the urinary nedaplatin concentration, hydrated rats have a lower concentration compared with nonhydrated rats. Hydration at the time of nedaplatin dosing could therefore be an effective strategy for minimizing nephrotoxicity.

We also examined the usefulness of prolonging the infusion time for reducing nephrotoxicity resulting from nedaplatin treatment<sup>69</sup>. This study provided evidence that prolonging the infusion time effectively minimizes the nephrotoxicity associated with nedaplatin. This effect may be related to a reduction in the maximal drug concentration in circulating blood, which consequently reduces the concentration of drug in the nephron. Because nedaplatin and cisplatin have similar nephrotoxic mechanisms, nedaplatin nephrotoxicity may be limited by cotreatment with antioxidants, although the capacity of antioxidants to ameliorate nedaplatin nephrotoxicity has not been studied.

### Usefulness of Animal Models of Renal Failure Using Differences Between Cisplatin- and Nedaplatin-Induced Lesions

Because cisplatin specifically injures the proximal tubule, several studies have used this drug to elucidate the pathophysiological mechanisms involved in the development of nephrotoxicity. The chronic cisplatin nephrotoxicity experimental model provides particularly useful insights into the detailed mechanisms underlying chronic renal failure and exhibits pathologic features similar to those seen with human lesions. Interestingly, the rat cisplatin-induced chronic nephrotoxicity model showed that interactions between different macrophage populations play an important role in the development of renal interstitial fibrosis<sup>70–73</sup>. Recently, it was reported that epithelial-mesenchymal transition of tubular epithelial cells contributes significantly to the development of renal fibrosis. The cisplatin nephrotoxicity model was used to confirm that prostaglandin E<sub>2</sub>, derived from activation of cy-

cloxygenase-1, may regulate renal epithelial regeneration via the prostaglandin E<sub>2</sub> receptor EP<sub>4</sub> through inhibition of apoptosis and epithelial-mesenchymal transition<sup>74</sup>. Comparatively little research has focused on the pathophysiology of chronic nephrotoxicity using the nedaplatin nephrotoxicity model. Further studies using this model may enhance understanding of the mechanisms leading to chronic renal failure and progressive fibrosis in humans, especially with respect to renal papillary injury with metaplastic changes in the papillary epithelium. These efforts may lead to the development of a novel therapeutic strategy for chronic renal failure in humans.

### Conclusions

Although reduced nephrotoxicity compared with cisplatin makes nedaplatin a promising second-generation platinum complex, this drug may also cause nephrotoxicity at doses that are therapeutic in humans, especially in the case of patients with deteriorated renal function. Histopathologically, nedaplatin nephrotoxicity in rats is characterized by apoptosis and/or necrosis, subsequent regeneration and cystic dilatation, not only in the proximal tubule but also in the distal tubule and the collecting duct. In severe cases, papillary necrosis also occurs. Comprehensive gene expression profiling revealed that nedaplatin nephrotoxicity might be induced by oxidative stress-mediated mechanisms. Furthermore, several genes have been identified as being specifically deregulated. Deregulation of the genes encoding cytokeratins 14 and 19 reflects the characteristic renal papillary injury associated with nedaplatin. Overall, recent research has provided significant insights into the molecular and histopathological events that are responsible for nedaplatin nephrotoxicity. These insights could lead to the establishment of an effective strategy for the safe use of nedaplatin in humans.

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