The Mechanism of Action and Clinical Transformation Evaluation of Antioxidant Drugs in Cisplatin-Induced Nephrotoxicity

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Abstract. In the current clinical practice of cancer, cisplatin has been established as the basic drug of chemotherapy for a variety of solid tumors with its exact efficacy. Although it is widely used, its nephrotoxicity seriously limits application and efficacy. Because oxidative stress is the core cause of cisplatin nephrotoxicity, antioxidant intervention is particularly important for reducing renal injury. In recent years, the protective effect of antioxidants such as N-acetylcysteine (NAC) on cisplatin nephrotoxicity has been confirmed in cell and animal experiments, but there are still many difficulties and challenges in its clinical transformation. This article reviews the core pathological mechanisms of cisplatin nephrotoxicity, including selective accumulation in the kidney, imbalance between oxidative stress and antioxidant defense, inflammatory cascade, and a variety of programmed cell death. On this basis, taking antioxidant NAC as an example, the mechanisms of its renoprotective effects were analyzed, and the current research progress was summarized. This paper provides a theoretical reference for the protective mechanism of antioxidants in cisplatin nephrotoxicity, and suggests the synergistic potential of combination drugs. However, the current research in this field still lacks high-quality standardized dosing regimens and high-quality clinical evidence. Future investigations can consider large-scale sample clinical trials, the establishment of individualized dosing schemes and the development of combination drugs, which can promote the transformation of basic achievements to clinical applications.

Keywords: Cisplatin nephrotoxicity; antioxidant; N-acetylcysteine; oxidative stress; inflammatory.

1. Introduction

As a chemotherapy option covering a large number of cancer species, the clinical application of cisplatin is extremely common [1-2]. In many standard chemotherapy regimens for malignant tumors, it is often defined as a first-line or cornerstone drug. However, the clinical utility of cisplatin is substantially constrained by its inherent toxicity, including nephrotoxicity, ototoxicity, gastrointestinal toxicity, bone marrow suppression, neurotoxicity, etc. The dosage of cisplatin is often limited due to side effects, the most important of which is nephrotoxicity. According to the clinical data, about 20% to 35% of patients have the risk of nephrotoxicity after taking cisplatin [2], which seriously restricts the play of clinical efficacy and the improvement of patients' prognosis. Cisplatin can cause renal toxicity through multiple molecular pathways, which can lead to acute kidney injury (AKI) or chronic injury [3]. AKI stands out as the most prevalent and severe manifestation of nephrotoxicity [3]. The concentration and reabsorption rate of cisplatin in renal tubular cells are 5 times higher than that in blood [3], which will lead to cisplatin accumulation in renal tubular epithelial cells. The accumulation of cisplatin will lead to a large number of reactive oxygen species (ROS), mitochondrial dysfunction and redox imbalance. These factors will further cause inflammation, cell death, cell apoptosis, and ultimately lead to AKI [4-5]. However, antioxidant drugs can eliminate excessive ROS and restore the function of antioxidant system, so as to reduce the damage of cisplatin to the kidney. Therefore, antioxidant intervention is one of the important strategies to reduce cisplatin nephrotoxicity.

Recent years have witnessed considerable research efforts, both basic and clinical, directed toward the protective effect of antioxidant drugs in renal injury. A large number of plant derived active molecules have been confirmed to significantly reverse or protect against cisplatin induced

nephrotoxicity, and its efficacy has initially reached clinical expectations [6]. These natural active ingredients cover a wide range of chemical categories, including flavonoids, saponins, alkaloids, polysaccharides and phenylpropanoid [6]. In addition, N-acetylcysteine (NAC), as a classic chemical synthetic antioxidant, has significant renal protective effect caused by cisplatin toxicity, which has been widely studied [7]. Although many antioxidant drugs have clearly shown protective effects on the kidney in cell and animal experiments, and do not affect the anti-tumor effect of cisplatin, their clinical transformation still faces considerable challenges. This article will summarize the pathological pathways underlying cisplatin nephrotoxicity, and take NAC as the main example to explore how antioxidant agents attenuate cisplatin-induced renal injury and evaluate their potential for clinical translation. Based on these analyses, this paper aims to improve the theoretical system of antioxidant protection against cisplatin nephrotoxicity, and provide feasible suggestions for subsequent related basic research and clinical application.

2. The Pathological Mechanism of Nephrotoxicity Caused by Cisplatin-Based Drugs

2.1. Selective Accumulation of Cisplatin in Kidney

After cisplatin enters the human body, about 65% to 95% of cisplatin interacts with circulating proteins, including albumin and transferrin, in the bloodstream [7]. Most of the free parts that do not bind to plasma proteins are excreted through the kidney [6]. Specifically, free cisplatin will first enter the urine through glomerular filtration and then undergo secretion and reabsorption at the renal tubular stage, which is also the reason for the high accumulation of cisplatin in the kidney [2].

Organic cation transporter 2 (OCT2) is one of the primary transporters mediating cisplatin uptake into renal tubular cells. *SLC22A2* gene is a structural gene encoding OCT2 protein, and its promoter hypermethylation will reduce the expression of OCT2 protein. This epigenetic change will significantly weaken the transcriptional activity of this transporter and directly affect the transport efficiency of cisplatin to the proximal tubule, thereby regulating the susceptibility of individuals to cisplatin nephrotoxicity [3]. In addition, if *SLC22A2* gene is mutated, cisplatin induced nephrotoxicity can also be alleviated. Cimetidine, as an OCT2 inhibitor, can protect the kidney by inhibiting the renal transport of cisplatin. Some natural products (e.g., formononetin) can also reduce the retention of cisplatin in renal tubules by inhibiting the function of OCT2 [2, 6].

In addition, copper transporter 1 (CTR1) and multidrug and toxin extrusion protein 1 (MATE1) are also involved in the transport of cisplatin. CTR1 promotes the entry of cisplatin into cells, while MATE1 is responsible for the transport of cisplatin from cells to the lumen. The imbalance of the two functions will lead to an increase of cisplatin accumulation in cells.

2.2. Oxidative Stress: Imbalance Between Reactive Oxygen Species Production and Antioxidant Defense

Oxidative stress is the core damage mechanism of cisplatin nephrotoxicity. Cisplatin accumulates in renal tubular epithelial cells and induces ROS production through a variety of ways. At the same time, it can inhibit the function of endogenous antioxidant system, break the balance between oxidation and antioxidant, cause lipid peroxidation and DNA lesions, triggering cellular dysfunction, which culminates in cell damage and the activation of apoptosis.

After cisplatin enters the cells, it first covalently combines with endogenous reduced glutathione (GSH) and other sulfhydryl containing molecules, resulting in a large amount of depletion of GSH, seriously weakening the first line of defense against oxidative damage [2]. Subsequently, cisplatin acts on mitochondria, inhibits the activity of mitochondrial respiratory chain complex I-IV, destroys mitochondrial membrane potential, leading to mitochondrial electron transport chain abnormalities [2,6], and then produces a large number of ROS such as superoxide anion (O2-), hydrogen peroxide (H2O2), hydroxyl radical (·OH) [7]. At the same time, cisplatin in the cytoplasm can also activate

NADPH oxidase (NOX) and further expand the source of ROS production. The damaged mitochondria will further release ROS, forming a vicious cycle of "oxidative stress mitochondrial damage" [5]. Moreover, because ROS can damage the mitochondrial respiratory chain and lead to ATP depletion [2], the collapse of energy metabolism makes renal tubular epithelial cells unable to maintain normal ion gradient and transport function, further aggravating the tendency of cell swelling and necrosis. Nrf2 is the core transcription factor that regulates mitochondrial function. It can maintain the dynamic balance of mitochondria and protect the ATP production ability of mitochondria. Therefore, Nrf2 activator dimethyl fumarate (DMF) can effectively reverse the ATP depletion caused by cisplatin, thereby reducing the mitochondrial damage caused by cisplatin [5].

2.3. Activation of Inflammatory Cascade

Cisplatin induced oxidative stress and cell damage products can effectively initiate innate immune and inflammatory responses by activating TLR4/MAPK/NF-κB/NLRP3 inflammatory cascade [8-9]. As shown in Fig. 1, molecular mechanisms are responsible for cisplatin-induced activation of the NLR family pyrin domain-containing 3 (NLRP3) inflammasome and the subsequent inflammatory cascade. Cisplatin can induce cell damage by producing ROS and promote the release of damage related molecular patterns (Damps); Damps act as an endogenous ligand to activate toll like receptor 4 (TLR4), and then simultaneously activate mitogen activated protein kinase (MAPK) and nuclear factor-κB (NF-κb) downstream signaling pathways; The two pathways synergistically induce NLRP3 inflammatory body assembly and activation, and ultimately promote the maturation and secretion of pro-inflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18), leading to inflammatory cascade amplification effect. This pathway is one of the core molecular mechanisms of cisplatin induced nephrotoxicity, ototoxicity and other organ toxicity.

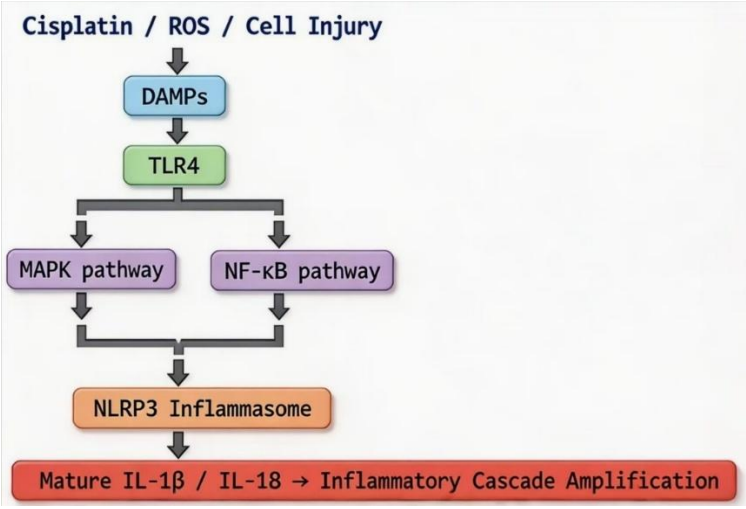


Figure 1. TLR4/MAPK/NF-κB/NLRP3 Inflammatory Cascade.

2.4. Apoptosis, Necrotic Apoptosis and Ferroptosis

In cisplatin induced nephrotoxic cells, apoptosis, necrotic apoptosis and ferroptosis are three types of programmed cell death with different molecular mechanisms and phenotypic characteristics. Cisplatin-triggered apoptosis in renal tubular cells is mainly through endogenous and exogenous pathways: in the endogenous pathway, mitochondria release cytochrome c, form apoptotic bodies with Apaf-1, initiate caspase-9, and then trigger caspase-3 to perform apoptosis; In the exogenous pathway, death ligands bind to death receptors, activate caspase-8, and then activate caspase-3 or trigger the endogenous pathway through bid [1]. Meanwhile, tauroursodeoxycholic acid (TUDCA) and other antioxidant drugs can reduce the cytotoxicity of cisplatin by inhibiting the apoptosis pathway [1]. Necrotic apoptosis is a kind of regulated programmed cell death, which is distinct from uncontrolled necrosis and apoptosis. Its core regulatory proteins are receptor-interacting protein kinase 1 (RIP1), RIP3 and mixed lineage kinase domain-like protein (MLKL). TNF-α activates tumor

necrosis factor receptor 1 (TNFR1) to trigger the formation of necrotic bodies, and then phosphorylates MLKL, leading to cell membrane rupture and necrotic apoptosis [1,3]. Ferroptosis is a newly discovered non apoptotic programmed cell death. Cisplatin can promote ferroptosis by consuming reduced GSH and inactivating glutathione peroxidase 4 (GPX4). At the same time, excessive iron can trigger the production of hydroxyl free radicals through Fenton reaction and induce lipid peroxidation [1].

3. The Mechanism of Action of the Antioxidant Drug NAC

As a classic thiol containing antioxidant, NAC's renal protective effect not only covers the classic antagonism of oxidative stress, but also includes inflammatory regulation, cell death intervention and other aspects. Oxidative stress is the core upstream mechanism of cisplatin nephrotoxicity. Cisplatin will consume a large amount of GSH in the process of renal metabolism. GSH is an important metabolic regulator in cells of the body and participates in the tricarboxylic acid cycle. Its active thiol group is an important functional group. It has pharmacological effects such as antioxidant stress, scavenging free radicals, inhibiting cell apoptosis and so on. It can effectively resist the related mechanisms of cisplatin nephrotoxicity, such as oxidative stress, cell apoptosis [10]. As a cysteine precursor drug, NAC can enter renal tubular epithelial cells through specific amino acid transporters because its structure is similar to amino acids, providing a rate limiting substrate for GSH synthesis, thus effectively reversing cisplatin induced renal GSH depletion. Moreover, because GSH can clear ROS, which acts as an essential initiator of NLRP3 inflammasome oligomerization and activation, NAC can also inhibit the downstream assembly and activation of NLRP3 inflammasome, reduce the mature release of IL-1 β and IL-18, inflammatory cascade amplification effect, break the positive and negative inflammatory loop of TLR4 and NLRP3, and finally inhibit the apoptosis and scorch of renal tubular cells [9]. In addition, NAC is a classical thiol donor, which can directly provide free thiol. On the one hand, it acts as a potent free radical scavenger, thereby interrupting the lipid peroxidation chain reaction [7]. On the other hand, free sulfhydryl can combine with the active hydrolysate of cisplatin, block the irreversible combination of cisplatin and the sulfhydryl of functional proteins in cells, protect the integrity of protein structure and function, and reduce the damage of renal tubular cells [7]. In terms of cell death intervention, NAC pretreatment can down regulate Bax, cleaved caspase-3 and other pro apoptotic proteins, and significantly reduce renal tubular cell apoptosis [8].

4. Research Progress on the Protective Effect of NAC Against Cisplatin-Induced Nephrotoxicity

4.1. Animal Model Experiment

In a study by GÜNTÜRK et al., the experimental rats were divided into four groups. They were control group (CONT), NAC group (NAC-250), cisplatin model group (CP), and cisplatin plus NAC group (CP+NAC). The renal injury model was established by intraperitoneal injection of 10 mg/kg body weight of cisplatin. After 4 hours of cisplatin administration, 250 mg/kg body weight of NAC was injected for 3 consecutive days [4]. The results showed that NAC at a dose of 250mg/kg had no adverse effect on the renal function of normal animals and was safe. Cisplatin administration can successfully induce severe acute kidney injury, which increases blood urea nitrogen and serum creatinine levels by about 7 and 8 times, respectively, compared with the control group. The combined intervention of NAC can significantly improve the renal function injury caused by cisplatin, in which the blood creatinine level decreased by nearly 50%, and the blood urea nitrogen level decreased by about 25%. The analysis of variance of the overall comparison among the four groups showed that the differences of the two indicators were highly statistically significant ($p=0.001$), confirming that NAC is an effective protective agent against cisplatin nephrotoxicity.

Histopathological examination further confirmed that cisplatin could cause severe hemorrhage, renal tubular epithelial injury and necrosis from the morphological level, and the protective effect of

NAC on cisplatin induced acute kidney injury. The results showed that the kidneys of animals given 250mg/kg NAC only showed very slight histological changes, which was not significantly different from the blank control group. However, the kidneys of cisplatin model group showed severe pathological injury, including significant renal tissue hemorrhage, extensive tubular epithelial cell injury and obvious tubular necrosis, and the pathological scores were significantly higher than those of the control group. NAC combined intervention can significantly reduce the above renal pathological changes, in which tubular necrosis is almost completely reversed, and the degree of hemorrhage and tubular epithelial cell injury are also significantly reduced.

4.2. Experimental Evidence for the Mechanism of Antioxidant Stress

From the perspective of mechanism, NAC can inhibit renal lipid peroxidation, improve the total antioxidant level and catalase activity, and block the excessive activation of oxidase induced by cisplatin [9]. As presented in Table 1 from Badr et al. Compared with the control group, the use of cisplatin can cause severe renal oxidative stress injury, such as the increase of lipid peroxidation level, but the total antioxidant substances and catalase activity decrease. After NAC intervention, the level of lipid peroxidation decreased significantly, while the total antioxidant substances and catalase activity increased significantly. These quantitative results confirmed that NAC could reduce the oxidative stress injury induced by cisplatin by inhibiting lipid peroxidation, enhancing antioxidant capacity and restoring catalase activity. The use of chlorogenic acid (CGA) intervention has a similar effect. If NAC is combined with CGA, the overall anti oxidative stress effect will be stronger.

Table 1. Effect of NAC and/or CGA on Cisplatin-Induced Changes in Renal Function Indices and Oxidative Stress [9].

Treated Groups	Lipid Peroxidation (nmol/mg Protein)	Total Antioxidant (mM)	Catalase (U/mg Protein)
Control	1.35 ± 0.18	2.18 ± 0.14	7.55 ± 0.61
Cisplatin	2.72 ± 0.24	1.24 ± 0.07	1.45 ± 0.57
NAC (250 mg/kg)	1.29 ± 0.06	2.11 ± 0.29	3.60 ± 0.57
CGA (20 mg/kg)	1.69 ± 0.14	1.70 ± 0.12	2.43 ± 0.09
NAC + CGA	1.35 ± 0.17	2.75 ± 0.48	3.25 ± 0.32
F, df, p value	12.22, 4, 0.0001	5.23, 4, 0.0069	29.14, 4, 0.0001

4.3. Experimental Evidence for Anti-Cell Death Mechanisms

According to the research results of Badr et al. cisplatin can mediate the extensive apoptosis of renal tissue cells, and its typical molecular characteristics are that the expression level of Caspase-3, a member of the executive caspase family, is significantly up-regulated, and the expression ratio of Pro apoptotic protein Bax and anti-apoptotic protein Bcl-2 is significantly increased. The above apoptotic effect is believed to be closely related to the damage of mitochondrial structure and function induced by ROS accumulation, which will further activate the endogenous apoptosis pathway [9]. In addition, the elevated expression level of TNF-α can also activate the extrinsic apoptosis pathway of cells by activating the death receptor signaling axis, which is another key pathway to participate in cisplatin induced apoptosis of renal tissue cells [9].

4.4. Research Progress of Combined Protection of NAC and Other Drugs

In the related research field of drug-induced renal injury protection, there has been a trend of transformation from traditional single antioxidant intervention to multi drug synergistic renal protection strategy in recent years. NAC combined with CGA can simultaneously block the activation of TLR4 pathway and the assembly of NLRP3 inflammasome, completely breaking the positive feedback loop of both [9]. As mentioned earlier, NAC can inhibit NLRP3 activation, but the injury signal can still continuously activate TLR4, and the loop cannot be completely cut off. Chlorogenic acid alone can block the upstream activation of TLR4 pathway and reduce the generation of NLRP3, but the existing ROS in cells can still activate the existing NLRP3. The combination of drugs is

upstream and downstream dual target blockade. Chlorogenic acid turns off upstream TLR4 and prevents the generation of new NLRP3 and inflammatory precursors. NAC shuts down the downstream NLRP3, preventing the release of mature inflammatory factors and in turn reactivating TLR4. It is equivalent to cutting off the two endpoints of the positive feedback loop at the same time. Inflammation can neither be initiated nor amplified, so it can completely break this vicious cycle. In addition, the joint intervention of NAC with various natural polyphenols and traditional Chinese medicine extracts is gradually becoming a new research direction in this field.

4.5. Deficiencies and Limitations of Current Research

First of all, most of the existing studies still remain in animal or cell experiments, and the clinical evidence is insufficient. The evidence is very consistent and the effect is clear second, at present, there is no globally recognized standard of "optimal dose, course of treatment and route of administration" uniformly recommended by authoritative guidelines. The research protocols differ greatly, the evidence is inconsistent, and no unified specification has been formed. At present, the clinical "relative consensus" is that intravenous administration is superior to oral administration, and the dose tends to be medium to high doses. However, because there are many differences between different people, such as age, renal function, OCT2 gene polymorphism, cisplatin dose, etc., the actual use of individualized drug administration is more necessary. Third, although many experiments and data have proved that NAC is safe, well tolerated and relatively safe in the treatment of cisplatin nephrotoxicity at conventional doses, there are still many drugs that do not have so much data support, and the safety of long-term use is not clear.

5. Conclusion

This article systematically reviewed the core pathological mechanisms of cisplatin induced nephrotoxicity, including the selective accumulation of cisplatin in renal tubular epithelial cells mediated by OCT2, Ctr1 and other transporters, oxidative stress caused by reactive oxygen species overproduction and antioxidant defense imbalance, TLR4/MAPK/NF-κB/NLRP3 inflammatory cascade reaction activation, and various programmed cell death pathways, including apoptosis, necroptosis, and ferroptosis. On this basis, this paper takes NAC as an example to explore the multiple mechanisms of its renoprotective effect, including glutathione supplementation, scavenging reactive oxygen species, inhibiting inflammasome activation and anti-apoptosis. The article also summarizes the protective effect of NAC in animal experiments and its synergistic potential with other drugs. Studies have shown that NAC can not only alleviate oxidative stress, but also interrupt the positive feedback loop of inflammation and enhance the body's total antioxidant capacity. The new idea of drug combination (e.g., NAC and CGA) is based on the theoretical foundation laid by these findings. This provides a research direction for the development of renal protection strategies based on antioxidant mechanisms in the future. However, the current research is mostly focused on the animal or cell level, lacking the support of large-scale and standardized clinical trials, and the optimal dosing regimen of NAC has not yet been unified, limiting its clinical promotion. In the future, multicenter, randomized controlled clinical trials should be carried out to clarify the optimal dose, course of treatment and route of administration of NAC. At the same time, explore the combined application strategy of NAC and other antioxidants or natural products, and promote the clinical transformation of individualized, multi-target renal protection programs.

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