MINI REVIEW

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Molecular mechanisms of cisplatin induced nephrotoxicity

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Received on: 05. 05. 2022

Revised on: 26. 05. 2022

Accepted on: 02. 06. 2022

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1. Background Nephrotoxicity associated with cisplatin (**CP**) utilization is considered as a great concerning hamper against the exploitation of CP clinical use **(Kotb *et al.*, 2021)**. Till now, there is still a struggle with the high incidence of nephrotoxicity associated with CP use. It was reported that about 25-35% of patients experienced a decline in glomerular filtration, accompanied by an elevation in blood urea nitrogen **(BUN)**, serum creatinine **(Scr)** levels that indicate the renal insufficiency which takes place upon CP single dose, despite the use of preventive techniques that mainly rely on the use of saline and hydration **(Gómez-Sierra *et al.*, 2018; Fang *et al.*, 2021)**. Further, it was reported that CP is associated with hypomagnesemia, which may persist for years upon its use **(Goren, 2003)**. Importantly, the kidney was delineated to be the most influential site for CP toxicity, particularly the S3 segment of the proximal tubules epithelial cells **(PTECs)**. Further, the concentration of CP in proximal tubules was identified to be roughly 5 times higher than that of it in the serum **(Amuthan *et al.*, 2021)**, regardless of all the accessible attempts that were adopted in order to prevent nephrotoxic effect of CP, around one third of patients on CP therapy still suffer from nephrotoxicity.

Keywords: Nephrotoxicity, cisplatin, molecular mechanisms

2. Pathophysiological mechanisms of nephrotoxicity induced by CP

Despite, full mechanism of CP nephrotoxicity is not fully elicited, multiple pathophysiological events were proposed to define the nephrotoxic effect of CP. These molecular mechanisms involve (1) cellular uptake of CP and its buildup into PTECs by the transport pathway **(McSweeney *et al.*, 2021)**, (2) metabolic conversion of CP into nephrotoxin thiol **(Zhang *et al.*, 2021)**, (3) Oxidative stress and mitochondrial dysfunction **(Santos *et al.*, 2007)**, (4) inflammation **(Manohar and Leung, 2018)**, (5) activation of the p38 mitogen-activated protein kinase (38 MAPK) **(Rashed *et al.*, 2011)**, (6) apoptosis **(Ozkok and Edelstein, 2014)**.

2.1. The contribution of OCT2 in the initiation of CP nephrotoxicity

Indeed, CP uptake into renal PTECs was previously identified as the driver of the CP nephrotoxic impact **(McSweeney *et al.*, 2021)**. The uptake of CP into renal cells is mediated through passive diffusion and transport system **(Peres and Cunha Júnior, 2013).** Organic cation transporters (OCTs) were implicated in the uptake and transportation of CP into the cells **(Koepsell, 2013;**

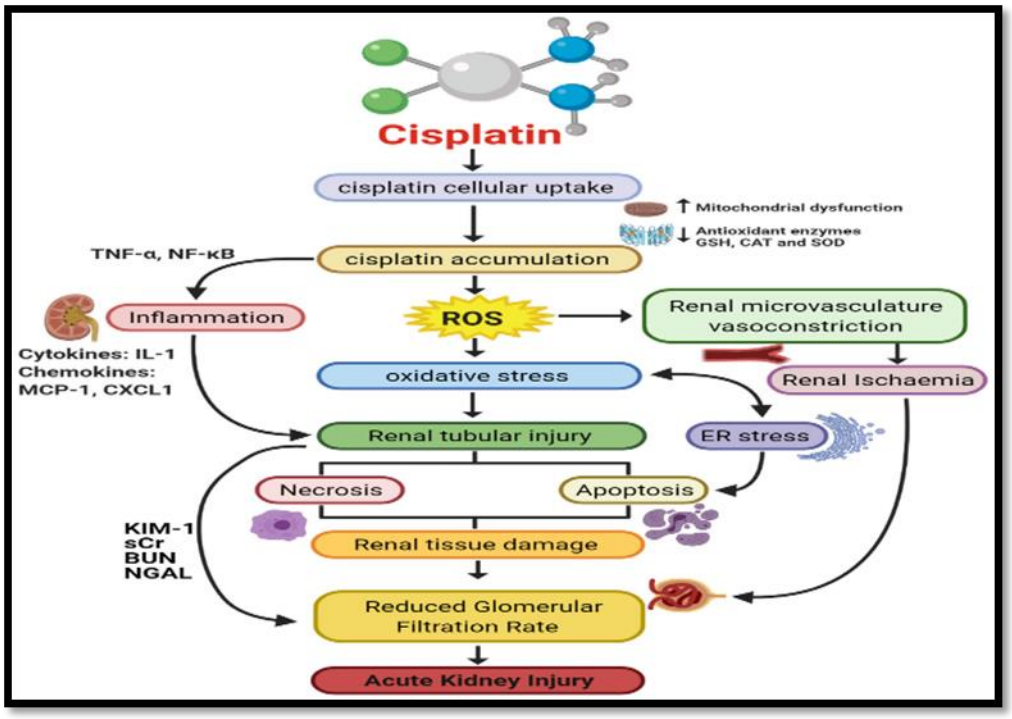

Figure 1. Schematic representation of the pathophysiological events of CP-induced nephrotoxicity (McSweeney *et al.*, 2021).

Zhang *et al.*, 2021). Three forms of OCTs were recognized in humans: OCT1, OCT2, OCT3. OCT1, the predominant OCT in the liver, OCT3, the key OCT in the placenta **(Koepsell *et al.*, 2007)**. OCT2 is expressed mainly in the kidney, particularly, the basolateral S3 segment of renal PTECs in both animals and humans **(Ciarimboli, 2012; George *et al.*, 2017)**. Importantly, OCT2 was observed to play a critical role in mediating-CP accumulation into PTECS; which in turn contributes mainly to the CP nephrotoxic effect **(Kim *et al.*, 2015)**. Besides, it was documented that 30% of the nephrotoxic effect associated with CP is related to OCT2 uptake **(Nieskens et al., 2018).** Further, it was evidenced by other studies performed on renal cells the higher toxicity of CP on the basolateral side than apical side linking it to the expression of OCT2 in this segment **(Ludwig et al., 2004; Gómez-Sierra et al., 2018).** Furthermore, OCT2-deficient animals exhibited a decrease in CP accumulation, which was verified by a reduction in the platinum buildup **(Soodvilai *et al.*, 2020)**. Moreover, pharmacological inhibition of OCT2 by other OCT2 inhibitors, such as cimetidine or imatinib, was demonstrated to have a nephroprotective effect, supported by the results that were indicative of improvement of kidney function, as well as the histopathological outcomings that have highlighted also the amelioration of the renal damage induced by CP **(Tanihara *et al.*, 2009; Katsuda *et al.*, 2010)**. Earlier study revealed that inhibition of OCT2 would have a nephroprotective effect without even compromising the antitumor properties of CP **(Sprowl *et al.*, 2013)**. Besides, diabetic rats were also found to be resistant to the CP-nephrotoxic impact, this is may be related to a downregulation in OCT2 expression in these rats **(Najjar and Saad, 2001; Grover *et al.*, 2004; Thomas *et al.*, 2004)**. Notably, CP analogues that include carboplatin and oxaliplatin were shown to be less nephrotoxic than CP, this is attributed to their inability to interact with OCT2 **(Ciarimboli *et al.*, 2005)**. Eventually, OCT2 represents an important target in the recent novel approaches that attempt to attenuate CP nephrotoxicity.

2.2. Metabolic conversion of CP into nephrotoxin thiol

Furthermore, gamma-glutamyl-transpeptidase enzyme, which located in the brush border of renal proximal tubules is involved in the metabolism of CP, converting it into the reactive nephrotoxin thiol, which formed upon conjugation of CP with glutathione (GSH) in the liver, contributing to its nephrotoxic effect **(Wainford *et al.*, 2008; dos Santos *et al.*, 2012)**.

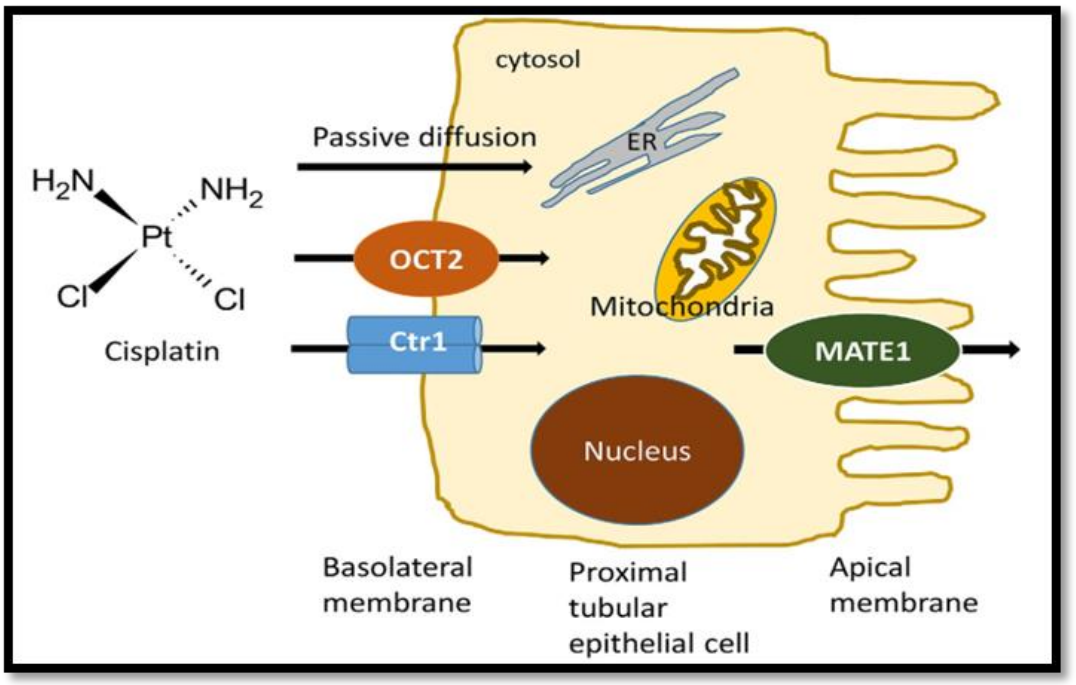

Figure 2. Schematic representation of OCT2-mediating transportation of CP into the PTEC (Zhang *et al.*, 2021)

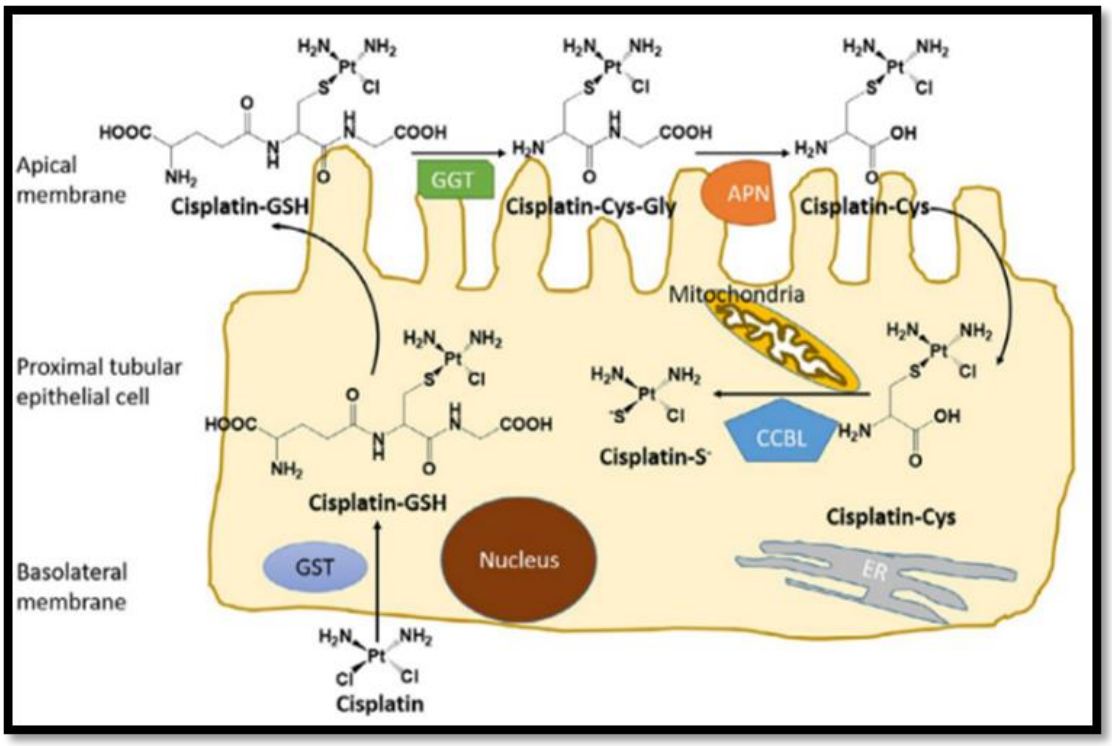

Figure 3. Illustration showing bioactivation of CP to its nephrotoxin (Zhang *et al.*, 2021).

2.3. Role of oxidative stress in the induction of CP nephrotoxicity

Oxidative stress was recognized as a vital contributor to the etiology of CP nephrotoxicity. CP triggers the surge of reactive oxygen species (ROS), through three mechanisms: first, during the conversion of CP into its highly reactive form, that is characterized by the rapid ability to react with thiol contained molecules like glutathione (GSH), ending up with the depletion and inhibition of antioxidant enzymes activity such as GSH, and superoxide dismutase (SOD), that lead to the disturbance in the balance of redox status homeostasis, ending with the accumulation of ROS and aggravation of oxidative stress status. Second, CP was implicated in the impairment of the respiratory chain of mitochondria. *In vitro* and *in vivo* studies performed on renal PTECS documented the inhibitory effect of CP on complexes I to IV of the mitochondrial respiratory chain. Further, they revealed the reduction in the level of intracellular ATP, affecting membrane electrochemical potential **(Satoh *et al.*, 2003; Santos *et al.*, 2007; Zsengellér *et al.*, 2012)**. Thirdly, ROS accumulation can also be mediated via the effect of CP on the cytochrome P450 (CYP450) system, that induces a rise in the level of catalytic iron, in turn leads to production of other potent oxidants **(Liu and Baliga, 2003)**. Eventually, all these events were involved in the CP-nephrotoxic effect and led to the generation of additional free radicals such as superoxide anion, hydroxyl radical, and hydrogen peroxide that induce a denaturation of renal cells components such as (lipid & protein, & DNA), and accretion of lipid peroxidation products malondialdehyde (MDA), contributing to the activation of additional multiple complexes signaling cascades that result in renal injury, inflammation and apoptosis.

2.4. CP-mediating inflammatory and cytokines activation:

The MAPK-signaling system is an interconnected serine/threonine kinases cascades, that responsible for regulation of vital important processes associated with cell differentiation, proliferation, survival as well **(Nguyen *et al.*, 2013)**. p38 is the critical MAPK, that was described its contribution in CP nephrotoxicity **(Yang *et al.*, 2019)**. Interestingly, authors have linked the initiation of p38 pathway to ROS release which mediated consequently to CP **(Tsuruya *et al.*, 2003)**. Besides, it was clarified that p38 activation would elicit in the production of tumor necrosis factor alpha (TNF-α), that is involved in CP-mediating renal inflammation **(Mishima *et al.*, 2006; Yano *et al.*, 2007)**. Importantly, p38 was suggested to be an important target in order to possess a protective effect on renal cell against CP nephrotoxicity **(Thongnuanjan *et al.*, 2016)**. In addition, the coordination of TNF-α was described in the activation of other proinflammatory cytokines like interleukin-2 (IL-2), interleukin-6 (IL-6) interleukin-β (IL-1β), chemokine monocyte chemoattractant protein-1(MCP-1), contributing to CP-inducing renal injury **(Ramesh and Reeves, 2002; Miller *et al.*, 2010)**. Previous study highlighted the importance of pharmacological TNF-α inhibitors and their related protective effects that are associated mainly with the decrease in the expression of these cytokines and chemokines, contributing to the amelioration of CP-mediating nephrotoxic effect **(Ramesh and Reeves, 2004)**.

Nuclear factor-kappa B (NF-κB) transcription factor, appears to be activated in response to CP-mediating surge of ROS , which in turn contributes to its translocation into the nucleus upon degrading its inhibitory protein IκBα, resulting in the activation of other robust inflammatory response, including TNF-α which itself plays a key role in the trigger of additional inflammatory related cytokines, contributing to recruitment of further inflammatory cells into the renal tissue **(Aggarwal *et al.*, 2004; Ramesh *et al.*, 2007; Sung *et al.*, 2008; Sánchez-González *et al.*, 2011)**.

CP-mediating cellular stress also seems to be participated in the release of myeloperoxidase (MPO), which is mediated by activated neutrophils. MPO is known to possess influential properties contribute importantly to inflammation and oxidative stress as well **(Loria *et al.*, 2008)**. Nowadays, MPO was suggested as an important contributor to CP nephrotoxicity **(Amirshahrokhi and Khalili, 2015)**.

In addition to ROS, nitrosative stress was also been in CP-induced renal injury **(Chirino *et al.*, 2008)**. It was demonstrated that there is induction in nitric oxide synthase (iNOS) synthesis consequently to CP administration; which results in an increase in nitic oxide production, that ends up with peroxynitrites formation, which has involved mainly in CP-mediating renal injury via reacting with superoxide anions **(Peres and Cunha Júnior, 2013)**. Notably, the excessive production of nitrosative stress and ROS would have a crucial

role in the initiation of other apoptotic cascades, which contribute to progressive renal injury and cell death **(Çetin *et al.*, 2006)**.

2.5. CP-mediating apoptotic nephrotoxic effect:

P53 was proposed to be an important mediator that contributes to the nephrotoxic effect of CP, evoking apoptotic renal injury. Following a DNA-damaging action mediated by CP, p53 activation occurs, resulting in cell cycle arrest. p53 exerts a role in activating pro-apoptotic Bcl-2 family protein p53 up-regulated modulator of apoptosis-alpha, which in turn contributes to the buildup of pro-apoptotic Bcl-2-associated X protein (BAX) in mitochondria and inhibition of anti-apoptotic protein B-cell lymphoma 2 (Bcl-2) as well, provoking a disturbance in equilibrium maintained between pro- and anti-apoptotic proteins. Besides, the liberation of cytochrome C in response to activated BAX is induced, eliciting activation of executioner caspase-3**(Yao *et al.*, 2007)**. Interestingly, several other reports have also linked the activation of p53 to the p38 MAPK pathway, associating it with CP-mediating apoptotic renal injury. In addition to p53-related pathways, the surge of ROS caused by CP has also been involved in the activation of BAX and its associated responses, in a p53-independent manner which also triggers caspase-3 activation and eventually ends with apoptotic renal cell injury **(Karasawa and Steyger, 2015)**.

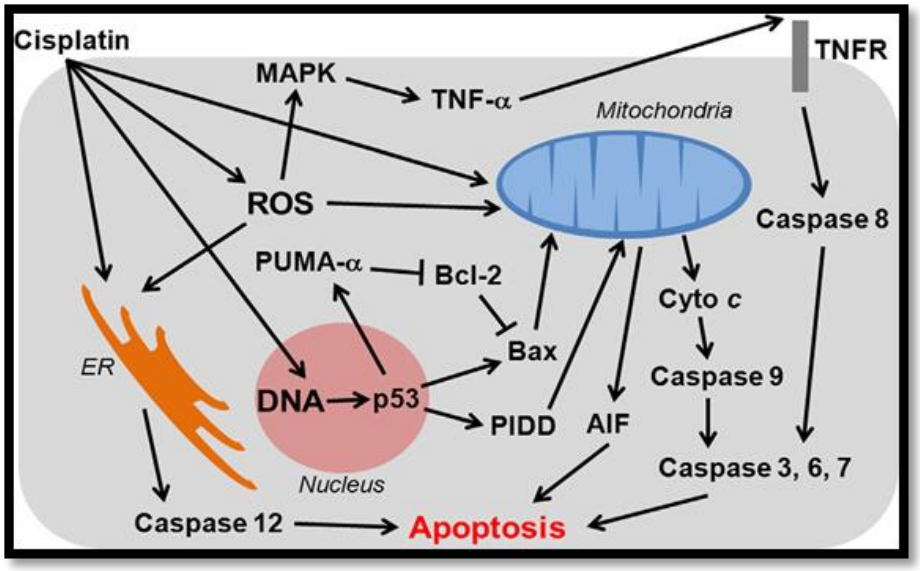

Figure 4. Schematic representation of CP- mediating apoptotic nephrotoxic effect (Karasawa and Steyger, 2015).

Conclusion

Accumulation of CP via OCT2 is considered as an important contributor to the enhancement of ROS release, which has a role in the exacerbation of oxidative stress status and the decrease induced in the antioxidant activity, eventually resulting in renal injury. Consequently, the trigger of inflammation is induced via activation of transcription factor NF-κB, promoting the production of several inflammatory cytokines such as TNF-α, IL-6, IL-2, and also activation of p38 MAPK, which in turn plays a role in the activation of other inflammatory and apoptotic pathways like p53, as well. Therefore, inhibition of OCT2 would have an ameliorating effect, suggested by a decrease in accumulation of CP into renal cells, attenuating oxidative, inflammatory, and apoptotic nephrotoxic effects of CP.

References:

Aggarwal BB, Takada Y, Shishodia S, Gutierrez AM, Oommen OV, Ichikawa H, Baba Y, Kumar A. Nuclear transcription factor NF-kappa B: role in biology and medicine. Indian J Exp Biol. 2004**;** 42:341-53.

Amirshahrokhi K and Khalili A-R. Thalidomide ameliorates cisplatin-induced nephrotoxicity by inhibiting renal inflammation in an experimental model. Inflammation.2015; 38 : 476-484.

Amuthan A, Devi V, Shreedhara CS, Rao V, Jasphin S and Kumar N. Vernonia cinerea regenerates tubular epithelial cells in cisplatin induced nephrotoxicity in cancer bearing mice without affecting antitumor activity. J Tradit Complement Med. 2021; 11: 279-286.

Çetin R, Devrim E, Kılıçoğlu B, Avcı A, Çandır Ö and Durak İ. Cisplatin impairs antioxidant system and causes oxidation in rat kidney tissues: possible protective roles of natural antioxidant foods. J Appl Toxicol. 2006;26:42-46.

Chirino YI, Trujillo J, Sánchez-González DJ, Martínez-Martínez CM, Cruz C, Bobadilla NA, Pedraza-Chaverri J. Selective iNOS inhibition reduces renal damage induced by cisplatin. Toxicol Lett.2008;176: 48-57.

Ciarimboli G. Membrane transporters as mediators of cisplatin effects and side effects. Scientifica. 2012;2012**:** 473829.

Ciarimboli G, Ludwig T, Lang D, Pavenstädt H, Koepsell H, Piechota H-J, Haier J, Jaehde U, Zisowsky J, Schlatter E. Cisplatin nephrotoxicity is critically mediated via the human organic cation transporter 2. Am J Pathol. 2005;167: 1477-1484.

dos Santos NAG, Rodrigues MAC, Martins NM and Dos Santos AC. Cisplatin-induced nephrotoxicity and targets of nephroprotection: an update. Arch Toxicol.2012;86: 1233-1250.

Fang C-y, Lou D-y, Zhou L-q, Wang J-c, Yang B, He Q-j, Wang JJ, Weng QJ. Natural products: Potential treatments for cisplatin-induced nephrotoxicity. Acta Pharmacol Sin.2021;42: 1951-1969.

George B, You D, Joy MS and Aleksunes LM. Xenobiotic transporters and kidney injury. Adv Drug Deliv Rev.2017; 116: 73-91.

Gómez-Sierra T, Eugenio-Pérez D, Sánchez-Chinchillas A and Pedraza-Chaverri J. Role of food-derived antioxidants against cisplatin induced-nephrotoxicity. Food Chem Toxicol. 2018; 120**:** 230-242.

Goren MP. Cisplatin nephrotoxicity affects magnesium and calcium metabolism. Med Pediatr Oncol. 2003; 41: 186-189.

Grover B, Buckley D, Buckley AR and Cacini W. Reduced expression of organic cation transporters rOCT1 and rOCT2 in experimental diabetes. J Pharmacol Exp Ther. 2004; 308: 949-956.

Karasawa T and Steyger PS . An integrated view of cisplatin-induced nephrotoxicity and ototoxicity. Toxicol Lett. 2015; 237: 219-227.

Katsuda H, Yamashita M, Katsura H, Yu J, Waki Y, Nagata N, Sai Y, Miyamoto K. Protecting cisplatin-induced nephrotoxicity with cimetidine does not affect antitumor activity. Biol Pharm Bull. 2010; 33:1867-1871.

Kim H-J, Park DJ, Kim JH, Jeong EY, Jung MH, Kim T-H, Yang JI, Lee GW, Chung HJ, Chang SH. Glutamine protects against cisplatin-induced nephrotoxicity by decreasing cisplatin accumulation. J Pharmacol Sci.2015;127:117-126.

Koepsell H. The SLC22 family with transporters of organic cations, anions and zwitterions. Mol Aspects Med.2013; 34 :413-435.

Koepsell H, Lips K and Volk C. Polyspecific organic cation transporters: structure, function, physiological roles, and biopharmaceutical implications. Pharm Res.2007; 24:1227-1251.

Kotb ES, Serag WM, Elshaarawy RF, Hafez HS and Elkhayat Z. The protective role of Gallic acid in Cisplatin nephrotoxicity. FSRT.2021.

Liu H and Baliga R. Cytochrome P450 2E1 null mice provide novel protection against cisplatin-induced nephrotoxicity and apoptosis. Kidney Int. 2003;63; 1687-1696.

Loria V, Dato I, Graziani F and Biasucci LM. Myeloperoxidase: a new biomarker of inflammation in ischemic heart disease and acute coronary syndromes. Mediators Inflamm. 2008; 2008:135625

Ludwig T, Riethmüller C, Gekle M, Schwerdt G and Oberleithner H. Nephrotoxicity of platinum complexes is related to basolateral organic cation transport. Kidney Int. 2004; 66; 196-202.

Manohar S and Leung N. Cisplatin nephrotoxicity: a review of the literature J Nephrol. 2018;31;15-25.

McSweeney KR, Gadanec LK, Qaradakhi T, Ali BA, Zulli A and Apostolopoulos V. Mechanisms of Cisplatin-Induced Acute Kidney Injury: Pathological Mechanisms, Pharmacological Interventions, and Genetic Mitigations. Cancers. 2021; 13; 1572.

Miller RP, Tadagavadi RK, Ramesh G and Reeves WB (2010). Mechanisms of cisplatin nephrotoxicity. *Toxins.2010;* 2; 2490-2518.

Mishima K, Baba A, Matsuo M, Itoh Y and Oishi R. Protective effect of cyclic AMP against cisplatin-induced nephrotoxicity. Free Radic Biol Med. 2006. 40; 1564-1577.

Najjar TA and Saad SY. Cisplatin pharmacokinetics and its nephrotoxicity in diabetic rabbits. Chemotherapy .2001;47; 128-135.

Nguyen LK, Matallanas D, Croucher DR, Von Kriegsheim A and Kholodenko BN. Signalling by protein phosphatases and drug development: a systems-centred view. FEBS J.2013; 280:751-765.

Nieskens TT, Peters JG, Dabaghie D, Korte D, Jansen K, Van Asbeck AH, Tavraz NN, Friedrich T, Russel FGM, Masereeuw R, Wilmer MJ. Expression of organic anion transporter 1 or 3 in human kidney proximal tubule cells reduces cisplatin sensitivity. Drug Metab Dispos.2018; 46:592-599

Ozkok A and Edelstein CL. Pathophysiology of cisplatin-induced acute kidney injury. Biomed Res Int.nal.2014; 2014 :967826.

Peres LAB and Cunha Júnior Add. Acute nephrotoxicity of cisplatin: molecular mechanisms. J Bras Nefrol**.** 2013; 35: 332-340.

Ramesh G, Kimball SR, Jefferson LS and Reeves WB. Endotoxin and cisplatin synergistically stimulate TNF-α production by renal epithelial cells. Am J Physiol Renal Physiol.2007;292: F812-F819.

Ramesh G and Reeves WB. TNF-α mediates chemokine and cytokine expression and renal injury in cisplatin nephrotoxicity. J Clin Invest. 2002;110;835-842.

Ramesh G and Reeves WB. Salicylate reduces cisplatin nephrotoxicity by inhibition of tumor necrosis factor-α. Kidney Int.2004; 65:490-498.

Rashed LA, Hashem RM and Soliman HM. Oxytocin inhibits NADPH oxidase and P38 MAPK in cisplatin-induced nephrotoxicity. Biomed Pharmacother. 2011; 65:474-480.

Sánchez-González PD, López-Hernández FJ, López-Novoa JM and Morales AI. An integrative view of the pathophysiological events leading to cisplatin nephrotoxicity. Crit Rev Toxicol. 2011; 41: 803-821.

Santos N, Catao C, Martins N, Curti C, Bianchi M and Santos A. Cisplatin-induced nephrotoxicity is associated with oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. Arch Toxicol.2007; 81: 495-504.

Satoh M, Kashihara N, Fujimoto S, Horike H, Tokura T, Namikoshi T, Sasaki T, Makino H. A novel free radical scavenger, edarabone, protects against cisplatin-induced acute renal damage in vitro and in vivo. J Pharmacol Exp Ther. 2003; 305: 1183-1190.

Soodvilai S, Meetam P, Siangjong L, Chokchaisiri R, Suksamrarn A and Soodvilai S. Germacrone reduces cisplatin-induced toxicity of renal proximal tubular cells via inhibition of organic cation transporter. Biol Pharm Bull.2020;43:1693-1698.

Sprowl J, van Doorn L, Hu S, van Gerven L, de Bruijn P, Li L, Gibson AA, Mathijssen RH, Sparreboom A. Conjunctive therapy of cisplatin with the OCT2 inhibitor cimetidine: influence on antitumor efficacy and systemic clearance. Clin Pharmacol Ther. 2013; 94: 585-592.

Sung MJ, Kim DH, Jung YJ, Kang KP, Lee AS, Lee S, Kim W, Davaatseren M, Hwang JT, Kim HJ, Kim MS, Kwon DY, Park SK. Genistein protects the kidney from cisplatin-induced injury. Kidney Int.2008;74:1538-1547.

Tanihara Y, Masuda S, Katsura T and Inui K-i. Protective effect of concomitant administration of imatinib on cisplatin-induced nephrotoxicity

focusing on renal organic cation transporter OCT2. Biochem Pharmacol. 2009;78:1263-1271.

Thomas MC, Tikellis C, Kantharidis P, Burns WC, Cooper ME and Forbes JM. The role of advanced glycation in reduced organic cation transport associated with experimental diabetes. J Pharmacol Exp Ther.2004; 311:456-466.

Thongnuanjan P, Soodvilai S, Chatsudthipong V and Soodvilai S. Fenofibrate reduces cisplatin-induced apoptosis of renal proximal tubular cells via inhibition of JNK and p38 pathways. J Toxicol Sci. 2016;41: 339-349.

Tsuruya K, Tokumoto M, Ninomiya T, Hirakawa M, Masutani K, Taniguchi M, Fukuda K, Kanai H, Hirakata H, Iida M. Antioxidant ameliorates cisplatin-induced renal tubular cell death through inhibition of death receptor-mediated pathways. Am J Physiol Renal Physiol. 2003; 285: F208-F218.

Wainford RD, Weaver RJ, Stewart KN, Brown P and Hawksworth GM. Cisplatin nephrotoxicity is mediated by gamma glutamyltranspeptidase, not via a CS lyase governed biotransformation pathway. Toxicology.2008; 249: 184-193.

Yang L, Yang Q, Li J, Hou G, Chen T and Ye M. Nephroprotective effects of Lachnum melanin against acute kidney injury induced by cisplatin in mice. Process Biochem.2019; 83: 198-205.

Yano T, Itoh Y, Matsuo M, Kawashiri T, Egashira N and Oishi R. Involvement of both tumor necrosis factor-α-induced necrosis and p53-mediated caspase-dependent apoptosis in nephrotoxicity of cisplatin. Apoptosis. 2007; 12: 1901-1909.

Yao X, Panichpisal K, Kurtzman N and Nugent K. Cisplatin nephrotoxicity: a review. Am J Med Sci.2007; 334:115-124.

Zhang J, Ye Z-w, Tew KD and Townsend DM. Cisplatin chemotherapy and renal function. Adv Cancer Res.2021;152: 305-327.

Zsengellér ZK, Ellezian L, Brown D, Horváth B, Mukhopadhyay P, Kalyanaraman B, Parikh SM, Karumanchi SA, Stillman IE, Pacher P. Cisplatin nephrotoxicity involves mitochondrial injury with impaired tubular mitochondrial enzyme activity. J Histochem Cytochem. 2012; 60:521-529.