Cisplatin Nephrotoxicity: New Insights in an Old Problem.

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Abstract.
Cis-diamino-dichloro platinum is one of the most widely used antineoplastic drugs. It is used therapeutically in 10-20\% of all cancers. It is active against a variety of solid tumors such as head, neck, lungs, ovaries, cervix, bladder, etc. The therapeutic use of the drug is combined with significant toxicities such as: neurotoxicity (85-95\%), ototoxicity (23-50\%), nephrotoxicity (30\%), gastrotoxicity and bone marrow suppression. Nephrotoxicity is a limiting condition since repeated episodes of acute kidney injury may lead to chronic kidney disease which then develops regardless of drug discontinuation. Unfortunately, nephrotoxicity and antineoplastic activity share common molecular mechanisms at the cellular level. Therefore, any attempt to reduce nephrotoxicity implies a reduction in the antineoplastic effect.

Continuing investigation of the mechanisms underlying cisplatin nephrotoxicity upon molecular level has provided an enormous amount of knowledge concerning accumulation of the drug in renal epithelial cells, cellular damage via destruction of cellular organelles and cellular proteins as well as induction of repair and rescue mechanisms such as autophagy toward the improvement of cell survival. All these metabolic pathways are currently candidates for therapeutic intervention. Moreover, new therapeutic approaches based upon natural derivatives such as phytochemicals are under laboratory investigation hoping to add more effective treatments in cisplatin nephrotoxicity.

In the present review we analyze the mechanisms of drug-induced cellular damage, the evidence so far, and developments in the effort to reduce nephrotoxicity without loss of drug activity.

Keywords: cisplatin, nephrotoxicity, autophagy, apoptosis, p53 protein, phytochemicals.
Introduction
The nephrotoxicity of cis-diamino-dichloro platinum (CDDP) is known from the very early steps of its clinical use and is a dose-dependent phenomenon that increases sharply when the dose exceeds 50 mg / m² / body surface area. It occurs late, about 10 days after administration of the drug, initially with a decrease in glomerular filtration rate (GFR) and then with an increase in serum creatinine. The incidence of acute renal failure (AKI) is estimated in 20-30% of patients receiving the drug.\(^1\) The lesion mainly concerns the proximal convoluted renal tubule (S3 section) and less the distal nephron. The reason for this peculiarity is that cisplatin accumulates inside the epithelial cell at a density five times that of blood, regardless of blood drug levels.\(^1\)

Key role plays the elimination of CDDP by the kidneys, either through filtration in the renal glomerulus or through active excretion from the renal epithelium. For active excretion, it enters the renal tubular epithelial cells through specialized Organic Cation Transporters (OCTs) on the basolateral surface of the cells and is excreted in tubular lumen via specialized anti-porters, in exchange with hydrogen ions, known as Multidrug and Toxin Extrusion proteins (MATEs).\(^2\)

The antineoplastic and cytotoxic action of the drug at the molecular level share common mechanisms and every effort to reduce nephrotoxicity reduces also its antineoplastic activity. Cytotoxicity of the drug is expressed via damage to nuclear and mainly to mitochondrial DNA, RNA and cell proteins. The above lesions lead to increased production of reactive oxygen species (ROS) and severe inflammatory reaction resulting in the activation of a variety of genes and proteins of the cell involved in the induction of apoptosis and cell necrosis.\(^3\) However, it seems likely that an intermediate stage between cell damage and the onset of apoptosis is the activation of the autophagy effect as a last effort for cell survival.\(^4\)

In advance we will try to examine individually the phenomenon of renal damage, the molecular mechanisms of transport and action of the drug upon cellular level and finally the possibilities of treatment of CDDP nephrotoxicity without reducing the antineoplastic effect of the drug.

Brief historical review
The first platinum salt complex with ammonium and chloride was discovered by the German chemist and physicist Heinrich Gustav Magnus in 1828 and consisted of an unusual platinum tetra-chloro-tetra-amino platinum polymer of the type \([\{\text{Pt} (\text{NH}_3)_4\} \text{PtCl}_4]\), it was green colored and became known as “Magnus' green salt”. Several years later, in 1844, the Italian chemist Michele Peyrone\(^5\) experimenting with the green salt of Magnus succeeded in synthesizing a new yellow salt called “Peyrone's yellow salt” which was the cis-diamino- dichloro platinum of the type \([\text{PtCl}_2 (\text{NH}_3)_2]\).

However, the antineoplastic properties of CDDP were discovered by chance much later, in 1961, by Barnett Rosenberg\(^6\) and his colleagues at Michigan State University, who were experimenting with the effect of electricity upon the growth of Escherichia coli. Knowing that platinum is a biologically inert material, they used platinum electrodes to conduct electricity to crops and were surprised to find that coliform bacterium colonies showed inhibition of bacterial cell division but continued to grow along the existing strains which acquired a filamentous texture. At first they believed that this was a result of the electric current, but when they changed the electrodes with another
metal, they found that the phenomenon was not repeated. Subsequent research revealed that the platinum anode electrode formed the octahedral salt of diamino-tetrachloro platinum as a result of oxidation by the ammonium chloride used as an electrolyte in the cultures according to the reaction: \( \text{NH}_4\text{Cl} + \text{Pt} \rightarrow [\text{PtCl}_4(\text{NH}_3)_2] \). They then studied the effect of diamino-dichloro platinum upon cancer cells and found that only the cis isoform of the complex had a strong antineoplastic effect. The results of the study were published in the journal “Nature” in 1965 and since then, the antineoplastic effect of the drug has been internationally established. Clinical use was approved by the FDA in 1978.6,7

### Nephrotoxic action of cisplatin

Acute renal failure is the most important clinical manifestation of CDDP nephrotoxicity. The onset of nephrotoxicity after cisplatin administration occurs relatively late, after about 10 days, and gradually resolves after 2-4 weeks. Its frequency is estimated in 20-30% of patients despite adequate preventive hydration. Clinically it is manifested by non-oliguric acute renal failure as an increase in serum urea and creatinine while maintaining the rate of urinary excretion. This is accompanied by salt and magnesium depletion and loss of urinary concentrating ability.3,8

The first report of nephrotoxicity dates back to 1971 originating from animal studies.3 Early clinical use of the drug was associated with acute renal failure in percentages ranging from 14-100%, depending on the cumulative dose.8 Predisposing factors for nephrotoxicity are: dose > 50 mg / m² / body surface area, cumulative dose, frequency of administration, age, female sex, hypoalbuminemia, liver cirrhosis, hypertension, alcohol intake, and pre-existing kidney damage. Diabetes mellitus is a protective factor for nephrotoxicity in experimental animals but has not been confirmed in humans.3,9

Hartman et al. showed that daily administration of cisplatin at a dose of 50 mg / m² for 5 days caused a significant reduction in GFR and hypomagnesaemia while administration of 20 mg / m² in the same manner had no effect upon GFR.10 The incidence of permanent renal failure defined as a prolonged decrease in eGFR > 25% of baseline is difficult to estimate and there are no large prospective studies with sufficient follow-up to provide conclusive results. A study from the Memorial Sloan Kettering Cancer Center9 reported an incidence of acute renal failure (AKI) of 31.5% and permanent renal failure, estimated as a permanent reduction in eGFR <29 ml / min / 1.73 m², at a rate of ~ 3%. This study included 821 patients and approximately half of them were followed for 5 years. It should be noted that no patient required dialysis, at least during the follow-up period.

Another recent study from an oncology center in Bellinzona, Italy, involving 184 patients over a 2-year period, without detailing age and follow-up time, showed transient reduction in eGFR > 25% of initial value at a rate of 40.2% and permanent kidney damage at a rate of 11.4%. In this study, important predisposing factor for permanent kidney damage was the administration of non-steroidal anti-inflammatory drugs (NSAIDs).11 Sasaki et al12 described two cases of acute renal failure that developed into permanent renal failure needing dialysis. They involved two men, aged 66 and 64, with esophageal cancer. They were given a combination of cisplatin and 5-fluorouracil, and developed AKI from the first dose of chemotherapy. Kidney biopsy showed mild damage to the proximal
convoluted tubule with a tendency to resolve but renal function did not recovered and patients remained in dialysis. In both cases a generic platinum product was administered.

After intravenous administration of cisplatin, the drug is removed from the blood in a three-phase manner. The first phase of halving the drug concentration (t1/2) lasts 20-30 minutes, the second phase lasts 60 minutes and the third phase about 24 hours. The first and second phases correspond to glomerular filtration and tubular excretion, and the third phase corresponds to the removal of the protein-bound drug. Two hours after administration, 90% of the drug is bound to proteins. Cisplatin accumulation is found increased in the liver, spleen and mainly in the kidney. Increasing the dose of the drug from 100 mg/m² to 200 mg/m² increases the initial phase of halving by 50% and probably explains the increased nephrotoxicity at higher doses.

Pathologically, cisplatin-induced acute renal failure is expressed first as acute tubular necrosis and then as interstitial fibrosis. Renal glomeruli are not affected. In experimental animals the lesion is located mainly in the S3 segment of the proximal convoluted tubule while in humans the lesions are predominantly located in the distal convoluted and collecting tubule.

Early functional disorders of the kidney include decreased renal blood flow (RBF) and GFR, both due to increased renal resistance. These disorders occur 2-3 days after Cisplatin administration. Early studies have shown that they are not prevented by administration of captopril or α-adrenergic blockers of the sympathetic nervous system (SNS). The interpretation given was that the reduction in renal blood flow is independent of the renin-angiotensin-aldosterone-system (RAAS) and SNS. Recent studies in experimental animals, WKY rats and spontaneously hypertensive rats, have shown that the increased renal resistance associated with nephrotoxicity of cisplatin may be due to stimulation of renal α1-adrenergic receptors, which are the most abundant in renal vasculature.

Early experiments in dogs from Daugaard et al. showed that cisplatin administration was accompanied within a few minutes to hours by reduced sodium and water reabsorption in the proximal tubule without concomitant change in RBF and GFR. At 48-72 hours after cisplatin administration there was significant reduction of RBF by 50% and GFR as a result of increased renal circulatory resistance. The authors also found reduced fractional reabsorption of sodium and water in the distal nephron implying that early damage to the proximal nephron extends to the distal nephron causing an increase in sodium and water excretion which is probably responsible for the polyuria that accompanies CDDP nephrotoxicity.

Therapeutic administration of cisplatin is accompanied by frequent electrolyte disturbances such as: hypomagnesaemia, hypokalaemia, hypophosphataemia, hypocalcaemia and hyponatraemia. The most important electrolyte disturbance is a dose-dependent renal magnesium loss with consequent hypomagnesaemia occurring in 40-90% of patients receiving cisplatin, while in patients receiving carboplatin occurs in about 10% only. Hypomagnesaemia is persistent and may last for up to 6 years after discontinuation of the drug. It is mainly due to renal magnesium loss and reduced absorption from the gastrointestinal tract, due to vomiting and diarrhea. Administration of other drugs such as loop diuretics, thiazide diuretics or alcohol consumption,
contribute to hypomagnesaemia. It appears that the increased magnesium losses from the kidney are due to the toxic effect of cisplatin upon the ascending limb of Henle’s loop and the distal convoluted renal tubule.\(^\text{16}\) Electrolyte disturbances that accompany cisplatin therapy should be recognized and treated promptly because in addition to their impact upon the quality of life, they may affect the efficacy and dosage of the drug, thus directly or indirectly endangering patient’s lives.

**Handling of cisplatin at the cellular level: Implications for cytotoxicity**

The entry of cisplatin into the intracellular space is performed in two ways: Either via passive drug diffusion through the cell membrane or via active transport through specialized organic cation transporters (OCTs). The ratio of passive diffusion and active transfer appears to be 1:1 for these two mechanisms.\(^\text{17,18}\) Passive drug diffusion requires the presence of the molecule in its aqueous solutions in the integral form of cis-diamino-dichloro-platinum \([\text{PtCl}_2(\text{NH}_3)_2]\). This specific form has a neutral electrical charge and can penetrate the cell membrane lipids following the concentration gradient. It is known, however, that chloride bonds with platinum are extremely sensitive. In aqueous solutions the molecule is hydrolyzed so that one or both chloride atoms are separated into chloride anions and replaced with an equal number of water molecules following the reaction:

\[
\text{PtCl}_2(\text{NH}_3)_2 + \text{H}_2\text{O} \rightarrow [\text{PtCl}_1(\text{H}_2\text{O})(\text{NH}_3)]^+ + \text{H}_2\text{O} \rightarrow [\text{Pt}(\text{H}_2\text{O})_2(\text{NH}_3)_2]^2+.
\]

The ionic form of the drug (hydro-ion) can no longer penetrate the cell membrane due to its electrical charge.\(^\text{18}\)

The dissociation of the cisplatin molecule depends upon the presence of chloride in the aqueous solution. Under normal conditions, and normal plasma chloride concentration (~ 110 mmol / L), 86% of the drug exist in the form of the dichloro-platinum, 11% in the form of the monochloro-platinum, and 4% in the ionic form of the hydrated platinum.\(^\text{17}\) It is obvious that under normal plasma chloride concentrations the neutral molecule of cis diamino-dichloro platinum can easily diffuse through the cell membrane into the intracellular space. However, the concentration of chloride in the intracellular space is much lower than in plasma (5-10 mmol / L) resulting in molecular conversion into its ionic bi-hydrated form. This conversion causes entrapment of the molecule in the intracellular space because it can no longer diffuse through the cell membrane and can only be actively excreted through specialized channels.\(^\text{18}\)

The active transport of cisplatin into the cell takes place through specialized channels located in the cell membrane. Some of them are responsible for the drug entrance and others for exit. These channels are not specific for cisplatin but instead carry a diversity of organic molecules with a cationic charge (Figure 1). Entry of cisplatin into the cell is achieved by two groups of transporters: Organic Cation Transporters (OCTs) and Copper transporters (Ctrs). OCTs appear to play a major role in the entry of cisplatin into somatic cells while Ctrs play a major role in cancer cells.\(^\text{2,19}\) Cisplatin is excreted from the cell via non-specific cation exchange channels for exchange with hydrogen ions known as Multidrug and Toxin Extrusion Proteins (MATEs) and ATPases 7A and 7B (ATP7A, ATP7B). ATPases mainly due to copper transport channels but also use cisplatin as a substrate. Mutations in the ATPase genes 7A and 7B are associated with Menkes and Wilson disease, respectively.\(^\text{2,19}\) OCTs are proteins of the Solute Carriers family.
(SLC), subfamily 22. The total number of subfamily members is 20 but the most common are the first three members: OCT1 (SLC22A1), OCT2 (SLC22A2) and OCT3 (SLC22A3). Of these, OCT2 is expressed in greater abundance in the kidney, especially its kidney specific isoform MATE-2K, while OCT1 is mainly expressed in the liver and OCT3 is mainly expressed in the heart. OCTs are expressed on the basolateral side of the secretory cell membrane and are the first step in transporting cations inside the cells. The driving force for their transport is the electrochemical difference of these cations on either side of the cell membrane which has a negative electrical potential in its intracellular side. This favors the concentration of platinum cationic derivatives inside the cell at a higher concentration than the plasma. OCT2 has a high affinity with cisplatin but also with newer platinum derivatives such as oxaliplatin and picoplatin.

![Figure 1](https://esmed.org/MRA/mra/)

**Figure 1:** Platinum transporters at the level of proximal convoluted tubule: Intracellular entry of platinum derivatives is accomplished from the basolateral membrane of the cell via the organic cation transporters (OCTs) and Copper transporter-1 (Ctrl-1). OCTs are the main transporters of the native cells whereas the Ctrl-1 is expressed mainly among cancer cells. Excretion of platinum derivatives to the tubular lumen is accomplished via the non-specific antiporters Multidrug and Toxin Extrusion proteins (MATE-1, MATE-2) and the kidney specific MATE-2K. These antiporters exchange organic cations with hydrogen ions from luminal space in a stoichiometry of 1:1. The affinity of these antiporters is high for oxaliplatin and lower for cisplatin which may explain the higher nephrotoxicity of cisplatin compared to oxaliplatin.

Attempts to inhibit OCT2 activity in order to reduce cisplatin nephrotoxicity have yielded conflicting results. Administration of the drug cimetidine, which antagonizes OCT2 activity in both experimental animals and humans, showed only partial nephroprotective effect. In a study included 18 patients with head and neck cancer in whom were administered cisplatin at a dose of 100 mg / m² and oral cimetidine at a dose of 800 mg, 30 minutes before cisplatin and 800 mg 90 minutes...
after the start of cisplatin infusion, the results showed that on the one hand cimetidine did not affect the pharmacokinetics of cisplatin in cancer cells and on the other hand that its nephroprotective effect was only partial. The authors' interpretation of the non-effect of cimetidine upon cisplatin pharmacokinetics is that OCT2 does not play a significant role in the uptake of cisplatin by cancer cells that may receive the drug via other transporters such as Ctr1 and the organic anion transporting polypeptide (OATP1B3) which are expressed in high density in cancer cells. Regarding the partial nephroprotective effect of cimetidine, the explanation proposed is that in addition to OCT2 antagonism cimetidine also reduces the activity of cisplatin exchangers MATE1 and MATE2-K, on the luminal surface of the epithelial cell, thus slowing the excretion of cisplatin in the urine and delays its presence in the intracellular space increasing the possibility of nephrotoxic action.

Another common drug that is widely used in clinical practice and appears to affect the nephrotoxicity of cisplatin is carvedilol. Published studies mainly concern experimental animals and not humans. In a study performed by Carvalho Rodrigues et al. in mice (male Swiss mice) implanted cancer cells (Sarcoma-180 cells) studied the effects of carvedilol (10 mg/kg for 3 days) and cisplatin (25 mg/kg on the 1st day). Carvedilol co-administration did not affect cisplatin cytotoxicity upon cancer cells and exhibited a significant nephroprotective effect. Concomitant administration of cisplatin/carvedilol showed lesser increase in urea and creatinine and lesser expression of toxic characteristics in renal biopsy specimens such as dilatation of the tubules and vacuolar cell degeneration. In the same study, the cellular mechanism of carvedilol nephroprotective effect was investigated and showed that oxidative stress was lower in the carvedilol group. This was not due to increased degradation of reactive oxygen species but to increased binding of ionic iron by the carvedilol molecule. The explanation given by the authors is that carvedilol contains in its molecule a carbazole group which binds ionic iron thus reducing the activity of Fenton reaction and so reduces the production of highly cytotoxic hydroxyl radicals.

In another study by Guo et al. performed also in experimental animals, mice (male C57BL/6J mice) they administered intraperitoneally carvedilol (2 mg/kg) and cisplatin (10 mg/kg) and studied the inhibitory effect of carvedilol upon cisplatin transporters OCT1-2 and MATE1-2K, and its nephrotoxic effect were evaluated. The nephrotoxicity was assessed by using the Kidney injury molecule-1 (Kim-1) as renal damage index and kidney biopsy evaluation. The results showed a stronger inhibitory effect of carvedilol upon OCT1-2 than upon MATE1 and MATE2. Co-administration of carvedilol and cisplatin in experimental animals resulted in a reduction in the transcription of the Kim-1 molecule to normal levels and the prevention of histologic findings of renal damage in renal biopsies. According to the authors the nephroprotective effect of carvedilol was due to the inhibition of OCT2 activity while leaving the activity of MATE1-2 almost unaffected resulting in reduced cisplatin entry into the cell without blocking its exit and hence reducing intracellular accumulation of the drug.

Upon clinical practice polymorphisms of the SLC22A2 gene encoding OCT2, such as SNP 808 G/T with resultant diminished activity of the transporter, have shown conflicting results, some studies showed...
nephroprotective effect against cisplatin nephrotoxicity while others did not confirm these findings. On the other hand, it appears that this polymorphism probably protects against the ototoxicity of cisplatin in the pediatric population.\textsuperscript{19}

Summarizing the above, it is obvious that any interventions at the level of cisplatin cell transporters provide only partial nephroprotection, probably because the drug enters the epithelial cells only by half via the active pathway.

**Cisplatin cytotoxicity at the molecular level**

The cytotoxic action of cisplatin has two components; the drug’s metabolic derivatives and the action of the drug itself. The formation of metabolic derivatives of cisplatin begins in the plasma with binding to glutathione under the influence of the enzyme glutathione-S-transferase. Cisplatin-glutathione complexes are filtered into the renal glomerulus and, via the enzyme γ-glutamyl transpeptidase (γ-GT) which is abundant on the surface of renal epithelial cells, are converted to cysteinylglycine complexes which in advance are metabolized to cysteine complexes via the enzymes aminopeptidases.\textsuperscript{3,23} Cysteine complexes enter the epithelial cells of the kidney and through the enzyme system of cysteine S-conjugate β-lyase are converted to thiols which are highly cytotoxic. Inhibition of these two enzyme systems does not affect the uptake of cisplatin by renal cells and reduces its nephrotoxicity, but inhibition of γ-GT has been associated with a reduction in the drug’s antineoplastic activity.\textsuperscript{3,23}

The action of the drug itself at the cellular level is pleotropic, inducing cell necrosis, cell autophagy, and cell apoptosis. The prevalence of each phenomenon depends on the drug’s intracellular concentration but also on the cell’s ability to activate rescue mechanisms and damage repair. The process of cell damage entails an exacerbation of oxidative stress and inflammation.

After cisplatin cellular entry, its molecular hydrolysis is promoted creating strong electrostatic molecules that form covalent bonds with nitrogen 7 (N7) of the purine bases (guanine-adenine) of DNA’s molecule.\textsuperscript{24} The bonds between purine bases are mostly located within the same DNA strand (intra-strand) and involve two adjacent guanine bases (~ 47-50%), one adjacent adenine to one adjacent guanine (~ 23-28%), or two non-neighboring guanines (~ 8-10%) either in the same DNA strand or between two complementary strands (inter-strand). These bonds result in inhibition of DNA’s transcription and replication, mainly mitochondrial but also nuclear. Inhibition of nuclear DNA transcription causes inhibition of cellular division and proliferation (cell-cycle arrest).\textsuperscript{24} Most vulnerable are the cells that show intense proliferation such as cancer cells, hematopoietic tissue cells and gastrointestinal epithelial cells. The immediate cellular response is activation of DNA’s repair mechanisms which are sufficient at the nuclear level but not at the mitochondrial level, rendering mitochondria extremely vulnerable to cytotoxic activity.\textsuperscript{3,24}

The cells of many malignant tumors have an excellent ability to repair their DNA and this is believed to be one of the causes of resistance to treatment with platinum derivatives. On the other hand, renal epithelial cells do not show increased reproductive activity and therefore should be more resistant to the cytotoxic action of cisplatin, but these cells show intense functional activity and increased mitochondrial density. Specifically, the epithelial cells of the proximal convoluted
The cisplatin molecule in its ionic form has the ability to react with many cellular elements such as DNA, RNA, proteins, phospholipids of cell membranes, cytoskeletal microfibers, actin, etc. The binding sequence of these elements favors DNA, followed by RNA, and then by proteins. Structural alteration of the above cellular elements causes a cascade of molecular reactions leading to severe cellular damage via organelle damage. The most vulnerable organelles to cisplatin cytotoxic activity are the endoplasmic reticulum and the mitochondria. Endoplasmic reticulum damage results from the accumulation of abnormally folded proteins. At the same time, cisplatin positively charged molecules accumulate inside the mitochondria, which present a strong electronegative charge, and drastically reduce ATP production, resulting in cellular starvation. These disorders result in increased production of reactive oxygen species and lead to mitochondrial oxidative stress and release of cytochrome-c resulting in the activation of proteins that promote cell apoptosis.

Cell apoptosis is the most common mechanism of cisplatin-induced cellular damage but the phenomenon of autophagy plays a role as well. Autophagy was described by Christian de Duve in 1963 who introduced the nomenclature “autophagy” from the corresponding Greek word “αυτοφαγία” which means “self-eating”. Autophagy is a normal cellular adaptive mechanism in conditions of metabolic stress or energy depletion. It is an intermediate stage in which the cell tries to avoid death by reusing damaged organelles and proteins, breaking them down into the basic building blocks. Activation of the autophagy effect suspends, at least temporarily, the apoptosis. In case of failure of autophagy to temporize the damage, the following stage is apoptosis (programmed cell death).

Experimental data showed that the induction of autophagy begins 2-4 hours after cisplatin application to cell cultures whereas cellular apoptosis begins after 8-12 hours. The tumor suppressor protein p53 is implicated in the regulation of autophagy. Sufficient evidence suggests that p53 and autophagy have a negative feedback relationship where p53 induces autophagy and, on the other hand, autophagy represses p53 activity. It seems likely that p53 induces the transcription of certain autophagy related genes such as damage-regulated autophagy modulator (Dram), which induces autophagy but it is also required for induction of apoptosis. Other autophagy genes activated by p53 are the Ulk1 and Atg7 genes (Figure 2).
Figure 2: Progress of autophagy: The start point of autophagy is characterized by the activation of the ULK1/2 proteins. Under normal circumstances ULK1/2 proteins are deactivated by the mTORC1/2 receptors which act as cellular energy receptors. In the case of starvation MTRC1/2 are deactivated and abolish suppression upon ULK1/2 proteins leading to indirect activation. On the other hand starvation activates also AMPK kinase which leads to direct activation of ULK1/2 proteins both of them leading to activation of autophagy cascade. Progress and integration of autophagy needs the activation of autophagy related genes and the production and activation of the relative proteins such as ATG3, ATG5 and ATG7. Activation of these proteins contribute to the formation and progression of autophagosome, collision with lysosome and final formation of autolysosome which eventually degrades and release to the cytoplasm all the engulfed material in the form of structural nutrients.

Once autophagy is activated, it provides nutrients to the cell, reduces oxidative stress, promotes DNA repair and suppresses adenosine monophosphate kinase (AMPK). All these events suppress p53 activation. It seems likely that the teleological purpose of this feedback is to protect the cell from damage under normal conditions of metabolic stress and reduces perpetuation of autophagy which predisposes to carcinogenesis.

In case the cell is unable to survive after the activation of the autophagy phenomenon, the next step of cell apoptosis follows. In general terms, this includes two mechanisms; the intrinsic (intracellular) which is activated under conditions of cell stress and DNA damage, and the extrinsic (extracellular) which is activated by extracellular factors. The intrinsic mechanism is characterized by mitochondrial damage and activates caspase-9. The extrinsic is characterized by activation of the death receptors located on the cell surface and comprising either the FaS-L / FaS-R proteins or the tumor necrosis factor-α receptor (TNFR1) and activates caspase-8. Downstream activation of executive caspases and especially caspase-3 is required to begin the end stage of apoptotic process. Both apoptotic mechanisms are characterized by
the activation of the p53 protein (Figure 3).\textsuperscript{30,31}

Under normal conditions, the cellular levels of p53 protein are very low because it is degraded by the proteasomes after binding to ubiquitin E3 via the MDM3 ligase. This process constitutes a mechanism of negative feedback of p53 levels in order to avoid the continuous activation of apoptosis.\textsuperscript{31} Under stressful cellular conditions, such as DNA damage, the transcription of the p53 protein is activated, which in turn leads to the immediate activation and transcription of about 500 genes while indirectly regulating the activity of many other genes. This highlights the p53 protein as a regulator of a variety of cellular functions and the most important activator of apoptosis.\textsuperscript{31}

\textbf{Figure 3:} p-53 protein and apoptosis: The crucial point of endogenous apoptosis is the activation of pore forming proteins BAX and BAK. These proteins are capable to aggregate in mitochondrial membrane and increase its permeability to cytochrome-c. Under normal circumstances BAX and BAK proteins are inactivated by the anti-apoptotic proteins BCL-XL, MCL-1 and BCL-2. These anti-apoptotic proteins can be inactivated by the pro-apoptotic proteins BH3 only proteins. p-53 protein regulates apoptosis by three pathways: 1. Activate the pro-apoptotic BH3 only proteins which leads to inactivation of the anti-apoptotic proteins BCL-XL, MCL-1 and BCL-2 and so leads to indirect activation of BAX and BAK proteins. 2. Direct activation of BAX and BAK proteins and 3. Promotes the association of APAF-1 with cytochrome-c which leads to the activation of caspase-9 subsequently activation of execution caspase-3 and eventually apoptosis of the cell.

Apoptosis of proximal tubular epithelial cells (PTECs) is initiated in a dose-dependent manner after exposure of these cells to cisplatin as it has been proven in vivo and in vitro studies. In a detailed experimental study Jiang M et al\textsuperscript{32} showed that exposure of PTECs to cisplatin concentration of 20 μM showed considerable apoptosis whereas exposure to concentrations varying between 50-100 μM showed apoptosis and necrosis. Generally higher concentrations of cisplatin favor cell necrosis. The same study showed that p53 protein activation
started after 2 hours of cell exposure to cisplatin and reached its maximum activity (3.5-fold increase) after 24 hours. Activation of caspase 3 was proportionally increased with the activity of p53 with a concomitant increase of apoptotic cells to by 68% after 24 hours incubation with cisplatin. By adding in cultures 100 μM of the caspases inhibitor, carbobenzoxy-Val-Ala-Asp-fluoromethyl ketone (VAD), caspase activation was completely abolished but apoptosis was partially reduced to 24.5%. On the other hand, adding 20 μM of pifithrin-α, the pharmacological inhibitor of p53 protein, caspase activation was inhibited to 50% and apoptosis was reduced to 27.5%. These results show that apoptosis of PTECs is initiated after p53 activation and the majority (~3/4) of apoptotic procedure is dependent upon caspases activation but there is also a proportion of apoptotic procedure which is not caspase dependent. Cummings BS et al showed also in previous studies that cisplatin induced apoptosis in PTECs was partially dependent upon caspases activation but there is also a proportion of apoptotic procedure which is not caspase dependent.

1. Hydration ≥ 3 L/day is strongly recommended. It is advised to administer serum normal saline (SNS 0.9%) in order to ensure diuresis > 100 ml/h. Administration of SNS is recommended because chloride anion reduces the production of positively charged cisplatin hydrated molecules and facilitates passive diffusion of cisplatin through the cell membrane.

2. Short hydration is weakly recommended (suggestion). It is applied only in an outpatient basis and in the case that the patient is capable to consume adequate nutrients and fluid from day 0 to day 3 of chemotherapy in an amount of at least 1.000 ml/day. Additionally 1-2 L of SNS 0.9% is administered i.v. in 4 hours duration before cisplatin administration. After that, cisplatin is administered in a 500-1.000 ml solution of SNS 0.9% in greater than 2 hours duration.

3. Diuretics administration is weakly recommended (suggestion). Osmotic diuresis by mannitol administration or the loop diuretic furosemide has been implicated in cisplatin nephrotoxicity prevention since 1970s in parallel with hydration. There is no convincing evidence of beneficial effect upon prevention of cisplatin nephrotoxicity but consider administration of mannitol in case of cisplatin administration ≥ 100 mg/m² or if the patient exhibits hypertension.

4. Administration of magnesium is weakly recommended (suggestion). The rationale for magnesium administration is that cisplatin administration can cause hypomagnesaemia as a consequence of renal and gastrointestinal loss of magnesium. Hypomagnesaemia is known to facilitate kidney injury and also increases OCT2 activity therefore correction of hypomagnesaemia may ameliorate kidney injury and suppress OCT2 activity. Although there are no large
scale randomized controlled trials to prove the efficacy of magnesium administration in the prevention of cisplatin nephrotoxicity, there are no side effects of magnesium administration. Despite the efforts to reduce cisplatin nephrotoxicity via adequate hydration and diuresis, it remains high and causes treatment failure in about 30% of patients receiving cisplatin. An effort has emerged in recent years among investigators to discover new treatments for cisplatin nephrotoxicity by using natural derivatives from plants with possible beneficial effect upon renal damage. These derivatives are called phytochemicals and encompass a broad spectrum of substances which are already tested in preclinical studies. There is a considerable number of phytochemicals under current experimental investigation either in laboratory animals or cultured cell lines. It seems likely that these compounds interfere with cellular mechanisms mediating oxidative stress, inflammation, energy metabolism and apoptosis. An interesting case of these compounds is maltol that interferes with a broad spectrum of intracellular pathways. Mi XJ et al showed in an experimental study comprising experimental animals, male ICR mice, and cellular toxicity model, human embryonic kidney 293 (HEK 293) cells, that administration of maltol 50 and 100 mg/Kg BW for 10 days ameliorated cisplatin induced toxicity. They specifically showed that animals treated with maltol exhibited lesser degree of kidney damage, oxidative stress and inflammatory reaction. These results became evident by reduction of kidney injury molecule-1 (KIM-1) and N-acetyl-d-glucosaminidase (NAG) excretion in urine. It was also showed a considerable reduction of glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) consumption in kidney tissue as well as diminished production of malondialdehyde (MDA). The authors also showed decreased apoptosis of renal cells and increased viability of HEK 293 cells by pretreatment with maltol. These events were consistent with an increase in phosphorylation of AMPK, PI3K and Akt kinase as well as modulation of mTORC phosphorylation. A significant decrease of p53 and BAX activation was also observed as well as decreased cleavage of caspase 3, 8 and 9 with concomitant increase of BCL-2 activation.

It is noteworthy that, if phytochemicals prove capable to interfere with such multitude of cellular mechanisms, they may be the future promise for cytotoxicity prevention of antineoplastic drugs, including cisplatin nephrotoxicity. In summary, after 6 decades of Cisplatin use in cancer chemotherapy it remains an effective drug for many solid tumors. Unfortunately a considerable number of patients (~30%) exhibit nephrotoxicity which limits the administration of this useful drug. Because nephrotoxicity and antineoplastic activity share common molecular mechanisms, it is difficult to reduce nephrotoxicity without reducing efficacy. Until now, there are no effective measures to overcome cisplatin nephrotoxicity. Continuing research upon the molecular basis of cisplatin nephrotoxicity as well as the use of phytochemicals may obtain favorable results in the near future.

Conclusions.
The most common feature of cisplatin nephrotoxicity is Acute Kidney Injury but repeated episodes of AKI may lead to Chronic Kidney Disease (CKD) and eventually End Stage Renal Disease. Accumulation of platinum derivatives in PTECs is higher than the concentration of...
the drug in the plasma. Entry of the drug in intracellular space is accomplished via two ways: passive diffusion through cell membrane and active transport through OATs in a ratio of 1:1 for each one.

Cytotoxicity of platinum derivatives is expressed either via cell necrosis or cell apoptosis. Higher concentrations of the drug favor cell necrosis whereas lower concentrations favor cell apoptosis. Cell apoptosis is usually preceded by the activation of cell autophagy. These two phenomena are regulated by the expression and activation of p53 protein in a complicated interplay. Therapeutic manipulations upon these two metabolic pathways are balanced at the edge of knife between cytoprotection and loss of anticancer activity.

Anticancer activity of platinum derivatives is mainly accomplished by forming DNA adducts with resultant cell cycle arrest. Most of malignant tumor cells possess a superior capacity of DNA repair mechanisms compared to somatic cells which may explain the acquired resistance of many malignant cells against platinum derivatives while any attempt to protect somatic cells decreases anticancer activity of the drug.

Adequate hydration mainly in the form of sodium chloride infusion is the only effective manipulation with considerable nephroprotective effect against cisplatin nephrotoxicity.

Newer therapeutic measures by the use of natural derivatives in the form of phytochemicals promise a better outcome of cisplatin nephrotoxicity in the future.

Acknowledgements
The authors thank Nicolaos Madias for his contribution to editing this manuscript.
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