REVIEW

HOW NEPHROTOXIC IS THE CANCER THERAPY IN CHILDREN?

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Abstract

Cancer mortality and morbidity rates in children are significantly higher, even long after cessation of treatment, than in the general population. Impaired renal function is one of the most severe observed late-effects after childhood cancer treatment. The current in depth review aims to underline the main features of the major drugs included in the chemotherapy regimens in children. For each of these drugs, we present the incidence and risk factors. The main pathophysiologic hypothesis of the drug nephrotoxicity, the clinical and biological features and the principal preventative and therapeutic methods are also presented. The associated comorbidities derived from nephrotoxicity are serious enough to consider the cancer prognosis as being unlikely optimistic. Further research is needed to provide less nephrotoxic drugs, to better identify renoprotective strategies and stratify the risk for nephrotoxicity.

Rezumat

Ratele mortalității și morbidității la copiii suferind de cancer sunt semnificativ mai mari, chiar și după încetarea tratamentului, decât în populația generală. Funcția renală afectată este unul dintre cele mai severe efecte târzii observate după tratamentul cancerului la copii. Această lucrare urmărește prezentarea principalelor caracteristici ale medicamentelor majore incluse în protocoalele de chimioterapie la copii. Pentru fiecare dintre aceste medicamente prezentăm incidența, factorii de risc, fiziopatologia nefrotoxicității, caracteristicile clinice și principale metode de prevenție și terapie. Comorbiditățile rezultate din nefrotoxicitatea tratamentului chimioterapic sunt suficient de grave pentru a considera un prognostic optimist al cancerului. Cercetări suplimentare sunt necesare pentru a furniza medicamente cu nefrotoxicitate mai mică, pentru a identifica noi strategii terapeutice renoprotectoare și a pentru a stratifica riscul de nefrotoxicitate.

Keywords: nephrotoxicity, cancer, children, chronic kidney disease

Introduction

Nephrotoxicity has been defined as the ability of an agent to cause structural kidney damage or functional impairment: glomerular and/or tubular dysfunction, impairment of blood pressure regulation and renal endocrine dysfunction. Nephrotoxicity in children with cancer depends on many factors including: the pre-existing renal damage due to the malignancy itself (by tumour infiltration or urinary tract obstruction), the patient's age and, respectively, the nature, duration and dosage of the nephrotoxic treatment. Chronic renal impairment in children with cancer may be due to a renal malignancy (e.g. Wilms tumour) or to the adverse effects of the treatment: chemotherapy, radiotherapy, surgery, immunotherapy or supportive treatment [92]. The chemotherapeutic agents can affect the kidney at different levels - glomerulus, tubules, interstitium, renal micro-vasculature - with clinical manifestations that range from an asymptomatic elevation of serum creatinine to acute renal failure (ARF) requiring dialysis [77]. Different forms of chemotherapy-induced kidney disease, like toxic acute tubular necrosis, thrombotic microangiopathy (TMA), crystal nephropathy, proteinuria/nephrotic syndrome, minimal change disease, focal segmental glomerulosclerosis (FSGS), membranous nephropathy, interstitial nephritis and tubulopathies have been reported [47, 48].

There are currently no standards to quantify the drug-induced kidney injury. Jimenez *et al*. [49] proposed a nephrotoxicity grading involving a score that combines the definition of acute kidney injury [1] and tubular dysfunction (expressed as

electrolyte disturbances) as follows: normal renal function (Grade 0); asymptomatic electrolyte disorders (hypomagnesemia, hypokalaemia or hypophosphatemia) and an increase in serum creatinine up to 1.5 times baseline value (Grade 1); need for electrolyte supplementation (magnesium, potassium, or phosphate) less than 3 months and/or increase in serum creatinine 1.5 to 1.9 times from baseline (Grade 2); increase in serum creatinine 2 to 2.9 times from baseline or need for electrolyte supplementation (magnesium, potassium, or phosphate) for more than 3 months after treatment completion (Grade 3) and increase in serum creatinine ≥ 3 times from baseline or renal replacement therapy (Grade 4).

Serum creatinine is an insensitive indicator of glomerular impairment that becomes elevated only when glomerular function is half reduced, thus underestimating the true frequency of nephrotoxicity. Glomerular filtration rate (GFR) is a better marker, much more sensitive and accurate, for the glomerular function [65, 92]. The chemotherapy drugs used in children with the highest nephrotoxic potency are ifosfamide and cisplatin. Both are widely used having a high efficacy in many solid tumours, but also causing chronic nephrotoxicity in 30 - 60% of paediatric cancer patients. Considering other cytotoxic drugs like carboplatin, methotrexate and nitrosoureas, renal toxicity is less frequent [92].

The current in depth review aims to underline the main features of the major drugs included in the chemotherapy regimens in children: alkylating chemotherapy agents (ifosfamide, cyclophosphamide), platinum agents (cisplatin, carboplatin), antimetabolites (methotrexate, clofarabine), nitrosoureas and other cytotoxic drugs (mithramycin, 5-azacytidine, anthracycline, actinomycin D). For each of these drugs, after a short introduction related to general information about the targeted drug, we present the incidence and risk factors (to highlight the importance of this topic as well as the correlation with age, dose, other comorbid factors), the main pathophysiologic hypothesis of the drugs nephrotoxicity, as well as the principal preventive and therapeutic methods. We did not approach in this review the nephrotoxicity secondary to radiotherapy, surgery, immunotherapy or supportive treatment.

Nephrotoxicity Due to Specific Cytotoxic Agents

Alkylating chemotherapy agents: Ifosfamide (IFO), Cyclophosphamide (CPA)

Ifosfamide is an important agent in the treatment of many paediatric solid tumours like soft tissue sarcomas, rhabdomyosarcoma and Ewing's sarcoma [27, 92]. Severe ifosfamide-induced renal damage has been reported also in patients with prior unilateral nephrectomy or kidney tumour infiltration [27, 85]. Ifosfamide nephrotoxicity may manifest with both acute and chronic glomerular as well as tubular impairment [91].

Incidence. Risk factors

A range of 1.4% - 30% of the children treated with IFO suffer some degree of renal impairment [8, 11]. A reduction in the glomerular filtration rate was reported in 50% of children studied for a median of 6 months after treatment with ifosfamide [90]. Hypertension has been reported in 5% of survivors [27, 92] and chronic renal failure (CRF) in 20 - 50% of children and adolescents after completion of treatment with ifosfamide [61, 91].

Children that received cumulative higher doses of ifosfamide (> 60 - 80 g/m^2) appeared to be at a greater risk, even though renal damage may occur in much lower doses [92]. There is a direct relationship described by Oberlin *et al*. between higher-dose and increased phosphaturia, reflecting greater tubular toxicity due to IFO administration [73], lower doses being associated with correlated less tubular toxicity [60].

Age has also been considered a risk factor, where severe toxicity was reported in infants and children younger than the age of 5, who may be more vulnerable to tubular toxicity [90]. The greater risk of kidney toxicity in younger children is attributed to renal ontogeny of the enzymes responsible for IFO metabolism. Higher levels of CYP3A, which metabolizes IFO to metabolite chloroacetaldehyde (CAA), have been observed in an animal model whose age corresponds to toddlerhood [6]. However, young age does not appear to be a predictor of long-term nephrotoxicity [93].

Other observed risk factors for nephrotoxicity related to chemotherapy were associated with cisplatin administration or, more importantly, unilateral nephrectomy. Children that had a nephrectomy showed an 11-fold increased risk of developing Fanconi syndrome after completion of chemotherapy with ifosfamide than those without it. Also the treatment with cisplatin in association with ifosfamide had a six-fold higher risk of developing phosphaturia and aminoaciduria [85].

Pathophysiology

Focal proximal tubular or tubulointerstitial changes with no or relatively low glomerular damage have been reported in renal biopsies after ifosfamide treatment [42, 92].

IFO enters the proximal tubule via the blood stream through the organic cation transporter 2 (OCT2) resulting in toxic CAA concentrations [18].

Nephrotoxicity caused by IFO is believed to be the result of oxidative stress caused by the metabolite CAA produced during its biotransformation in the kidney.

CAA shares metabolism with Cytochrome P450 2E1 (CYP2E1) and xanthine oxidase which leads to reactive oxygen species (ROS) production, which

targets the mitochondria and the lysosomes and determines membrane damage. Lysosomal Haber-Weiss reaction and the disruption in the respiratory chain of the mitochondria lead to hepatotoxic effects. Antioxidant glutathione (GSH) has shown to be protective against these negative effects [72].

Similar to the liver, the kidney is capable of detoxifying toxic agents (such as locally produced chloroacetaldehyde) through GSH, unfortunately it is greatly decreased in the kidney as compared to the liver, reason for which the nephrotoxic effect of CAA is much greater at renal level. The kidney possesses the enzymes responsible for the metabolism of IFO and, lately, it has been shown to be capable of producing levels of CAA, levels which surpass the detoxification rate [47, 48].

Both CAA and reactive oxygen species (ROS) may damage cellular proteins and DNA, and produce changes in intracellular sodium and calcium concentrations leading to impaired solute reabsorption and necrosis [17, 27].

The production of CAA may explain why ifosfamide, but not cyclophosphamide causes nephrotoxicity, since cyclophosphamide, is not transported *via* OCT2 and thus it is unable to produce the same nephrotoxic events [76].

Clinical and biological features

The acute proximal tubular impairment leads to hypophosphatemia due to phosphaturia. The less specific features of ifosfamid tubular nephrotoxicity are elevated urinary excretion of renal tubular enzymes. Glomerular impairment leads to proteinuria (particularly albuminuria), reduced glomerular filtration rate (GFR) (studies showed a lower mean GFR in IFO treated children`s group) [25, 92, 107], glucosuria, aminoaciduria, phosphaturia, and bicarbonaturia. Tubular damage appears to be the primary event leading to compromised glomerular function however the induced renal injury of IFO is presently mainly attributed to CAA inhibition of NADH: oxido-reductase activity [25, 97, 104]. If tubular damage is the first manifestation of renal toxicity determined by IFO, progressive glomerular damage appears usually at distance from the treatment in the follow-up periods [92].

Both ifosfamide and cyclophosphamide might be responsible for haemorrhagic cystitis. These drugs have a corrosive liver metabolite - acrolein - that is filtered by the kidneys and accumulates in the bladder where it damages the urothelium causing ulceration and exposure of underlying muscularis mucosa and vasculature. Both cyclophosphamide and ifosfamide can also cause the syndrome of inappropriate antidiuretic hormone secretion (SIADH) and nephrogenic *diabetes insipidus*. Chemotherapy-induced nausea may also play a contributory role, since nausea stimulates the release of ADH. Chronic ifosfamide-induced nephrotoxicity in children may result in hypophosphatemic rickets (HR), renal tubular acidosis (RTA), renal glycosuria (in the absence of hyperglycaemia) leading in severe cases to Fanconi syndrome [91]. CRF, HR and RTA may cause growth impairment in children treated with IFO [24].

Pharmacokinetics and toxicology

Alkylating chemotherapy agents' pharmacodynamics is based on the ability to their ability to alkylate DNA which in return leads to cytotoxicity and cell death.

Ifosfamide, the structural isomer of cyclophosphamide, was developed as a broader alternative to cyclo-phosphamide about fifty years ago, and although the period of usage was a long one, the study of ifosfamide pharmacokinetics is still under development.

The active form of isofosfamide is an alkylating drug that has one chloroethyl group. Although the metabolization happens primarily in the liver, the route of administration appears to influence the metabolism. Oral administration leads to increased IFO metabolites levels, like those demonstrated to be present in paediatric patients CSF [32]. Also, the oral route of administration together with the duration of administration are linked directly to renal impairment [4].

Out of the toxicity effects noted, the most important ones are neurotoxicity, myelosuppression, and last but not least glomerular dysfunction and global impairment of proximal tubule function. The excretory system modifications are most likely due to the effect of cloroacetaldehyde over the levels of carnitine in the renal tubules [32], carnitine being a modulator of nephrotoxicity by influencing the oxidative and nitrosative apoptotic signalling [87].

Nephrotoxicity is the dose-limiting toxic effect of IFO, this propriety being given by acrolein's (the urotoxic metabolite of IFO) elimination in the urine.

Cyclophosphamide is an anticancer drug activated by the cytochrome P450 enzymes, of the oxazaphosphorine class. Due to the adverse side-effects, after many decades of study the pharmacokinetics and toxicology of this substance, studies are still conducted with the purpose of overcoming the dose-limiting adverse reactions of CPA [104]. The administration is preferably oral with a peaking concentration at 1h after administration. The metabolism of CPA is mainly in the liver and is more rapid in children, being mainly secreted in the urine. Unlike IFO, the neurotoxicity and nephrotoxicity of CPA is limited, being used in high doses for treatments in paediatric patients [109]. CPA nephrotoxicity is mainly controversial for patients with impaired renal function [40]. The highest CPA toxicity is expressed in myelosuppression but haemorrhagic cystitis and cardiac toxicity are also important. In adult patients, liver injury due to acrolein was present and also thrombotic events in female patients but no sex

based differences were noted in the severity of renal toxicity [30].

Prevention. Treatment. Prognosis

The risk of ifosfamide nephrotoxicity might be reduced if cumulative doses over 80 g/m2 could be avoided, particularly in children younger than 5 years [62] and patients that have previously received cisplatin or patients with altered renal function [91]. It appears that concurrent administration of N-acetylcysteine (NAC) [17], melatonin [89] or glutathione [5] may reduce ifosfamide nephrotoxicity due to an antioxidant effect [39]. The kidney toxicity determined by IFO is due to oxidative stress, which can be developed due to GSH depletion. While antioxidants would address the ROS detoxification acting as nucleophiles, some of them (NAC) also address to the depleted GSH levels by increasing cysteine which is vital for its formation [39].

In case a high-dose of intravenous cyclophosphamide is used, high fluid load should be administered to prevent haemorrhagic cystitis [65]. Although mesna (a synthetic thiol compound capable of detoxifying potentially nephrotoxic ifosfamide metabolites) is useful in the prevention of haemorrhagic cystitis induced by ifosfamide and cyclophosphamide, there is no evidence that it reduces the risk or the severity of nephrotoxicity.

The treatment of nephrotoxicity induced by these alkylating agents may include renal supportive care such as dialysis and/or renal transplant. In case of hypophosphataemic rickets, supplementation with phosphate and bicarbonate in growing children is needed [27, 91, 97].

Proteinuria and blood pressure should be monitored regularly, and in case of significant proteinuria, association of an angiotensin-converting enzyme inhibitor or angiotensin II blocker should be considered [9].

The degree of reversibility of chronic ifosfamide nephrotoxicity appears to be unpredictable, but some authors have associated in adult patients treated with ifosfamide when at paediatric age, kidney damage that was proven irreversible and has been compared with a unilateral nephrectomy, the extent of the damage being correlated directly with the cumulative doses [4].

The tubular and glomerular toxicity have shown no significant improvement over time, on the contrary, it may continue to deteriorate even after ifosfamide has been stopped. Two long term follow up studies demonstrated that ifosfamide nephrotoxicity may persist for at least 10 years after completion of chemotherapy [73, 92].

Platinum agents: cisplatin (CDDP or CIS), carboplatin

Cisplatin (CIS) has a well-defined role in the treatment of several paediatric solid malignancies like brain tumours, osteosarcoma, neuroblastoma, germ-cell tumours and liver tumours [92]. It accumulates in the kidney at higher concentrations than in the blood and other organs, thereby contributing to kidney injury [9], causing both acute and chronic glomerular and tubular toxicity [49, 91]. However the most frequent manifestation of cisplatin`s nephrotoxicity is acute kidney injury (AKI) [67]. Renal damage caused by cisplatin is typically persistent in children, being detectable in blood up to 20 years following treatment [36, 92].

Carboplatin has been used as an alternative to cisplatin in several solid tumours (e.g., brain tumours, neuroblastoma or germ-cell tumours) with apparently high efficacy and lower nephrotoxic effects than cisplatin. Carboplatin nephrotoxic consequences are usually milder, less frequent, and often reversible [24, 92, 97].

Incidence. Risk factors

The reported incidence of cisplatin glomerular toxicity varies from 10 to over 80% depending on the timing of investigation. Hypomagnesaemia occurs in 30 to 100% [49, 92] and AKI in 20 - 30% of patients [49]. In the Jimenez' study [5], hypophosphatemia (using the KDIGO age-dependent phosphate values) [38], was more frequent (65%) and persistent than hypomagnesaemia (40.7%) in cisplatin treated children. It was reported that 60 to 80% of these children may develop CKD (stage $\geq$ 2) [27, 91, 92].

The incidence of glomerular impairment due to carboplatin use, ranges from 0 to 15 - 25% and that of hypomagnesaemia from 0 to 10% [11, 92]. Long-term glomerular impairment induced by carboplatin appears to be more common in children with higher ages [93].

Cisplatin clearance by the kidney depends upon the glomerular filtration and tubular secretion. There is an apparent dose - dependent risk: moderate or severe glomerular impairment (low GFR) and hypomagnesaemia (tubular toxicity) at 1 - 2 years post-treatment are more frequent in children receiving a high cisplatin dose (> 40 mg/m^2/day) than in those receiving a lower dose (40 mg/m^2/day) [92, 94].

Other risks or exacerbating factors include: cumulative doses, the frequency of drug administration, dehydration, previous exposure to cisplatin, pre-existing kidney damage, hypoalbuminemia [27], concomitant use of other nephrotoxic drugs (ifosfamide and methotrexate, but also other nephrotoxic agents such as amino-glycosides, NSAID or iodinated contrast media). When cisplatin is administered with bleomycin or gemcitabine, it may cause thrombotic microangiopathy (TMA) mainly due to direct endothelial injury with secondary platelet activation [65, 103].

For children treated with carboplatin, the frequency and severity of induced hypomagnesaemia appears to be related to cumulative dose and an older age at treatment initiation [10]. Other risk factors for carboplatin nephrotoxicity are the association with other potentially nephrotoxic agents (e.g. cisplatin,

ifosfamide, melphalan) and/or pre-existing renal injury [29, 91].

Pathophysiology

Cisplatin is transported via organic cation transporter 2 (OCT2) and to a lesser extent, CTR1 transporters, and thus it enters renal epithelial cells *via* the OCT2. Animal studies suggest that hypomagnesemia enhances cisplatin accumulation in renal tissue by upregulating the OCT2 [108]. The uptake of cisplatin by OCT2 results also in activation of signalling pathways (mitogen-activated protein kinase (MAPK), P53 and possibly P21), leading to renal tubular cell death. At the molecular level, CDDP damages the nuclear and mitochondrial DNA, inducing the production of reactive oxygen species (ROS) and subsequently leading to activation by apoptosis or necrosis. Oxidative stress appears to be both the driving force and end result of some of these changes [23, 107].

CDDP nephrotoxicity may be enhanced by an inflammatory reaction produced by the activation of proinflammatory cytokines and chemokines, among which TNF-alpha appears to play a central role [84].

Carboplatin is less nephrotoxic due to the fact that the chloride in the cis position is replaced by carboxylate or cyclobutane, respectively. In addition, organic cation transporter OCT2 does not transport carboplatin. Hypomagnesaemia appears to be the most common manifestation of carboplatin nephrotoxicity, but it occurs less often than with cisplatin [65].

Clinical and biological manifestation of nephrotoxicity

In contrast to ifosfamide, cisplatin-induced tubular damage leads to magnesuria and thus secondary hypomagnesaemia [91, 97]. Hypomagnesaemia may cause paraesthesia, tremor, tetany and convulsions confirmed by experimental models where it exerted CNS depressant activity [98] or affects protein synthesis, and neuromuscular excitability [7]. Chronic magnesuria, hypomagnesaemia, hypocalciuria, with normokalaemia or mild hypercalcemia may result from dissociation of magnesium and calcium due to tubular lesion [92]. On the other hand, if hypomagnesaemia occurs, the release of parathyroid hormone is inhibited, leading to potentially severe hypocalcaemia (although these occur less commonly) [19, 38]. Tubular toxicity may also result in mild hypokalaemia metabolic alkalosis, aminoaciduria, glycosuria, phosphaturia and increased urine excretion of retinol binding protein (RBP), β2 microglobulin, N-acetylglucosaminidase, alanine aminopeptidase and β-galactosidase [92] and distal renal tubular acidosis [67]. Renal concentrating defect and polyuria was also observed, being produced most probably due to distal nephron resistance to vasopressin [59, 67].

Other renal manifestations include salt wasting [67], a Fanconi-like syndrome [74], anaemia (due to erythropoietin deficiency) [56], chronic renal failure [54] and TMA [12].

Hypertension may occur, most likely by way of renal or/and vascular toxicity [72, 91, 92]. Patients with nephrotoxicity may develop impairment in longitudinal growth, the height being the most affected. The impairment in growth may be due to phosphate and magnesium urinary losses [49].

Clinical sequelae of carboplatin nephrotoxicity in children are rare and usually fully reversible, except for hypomagnesaemia [93]. Carboplatin use usually does not affect glomerular filtration [13] or may produce just a small reduction in GFR [24]. ARF and occasionally CRF have been reported in children treated with high-dose carboplatin [29]. Tubular damage leading to hypomagnesemia may occur [28] but it is usually reversible [25], the same like natriuria and hyponatremia that have been occasionally described [100].

Pharmacokinetics and toxicology

CDDP and carboplatin are platinum II complexes with two ammonia groups present in the cis position. The cytotoxicity mechanism of cisplatin and carboplatin is the same, they become aquated and by binding to the DNA they form lesions. DNA is the preferred target for all platinating agents [37].

CDDP has several side effects like nausea, vomiting, neurotoxicity, myelosuppression and ototoxicity. The dose-limiting effect however is the nephrotoxicity that accompanies the treatment [96].

Although, the doses are followed by a wash-out period of 5 days after each administration, the effect of cisplatin seems to be cumulative. The mechanisms of action, as stated before, are still in debate. Apoptosis and necrosis of the tubular cell through cisplatin activated signalling pathways is one presumed mechanism [96]. Additionally, the unbound to proteins CDDP is excreted by glomerular filtration. The accumulating CDDP in the tubular cells contributes to stimulation of inflammation and to an increase in reactive oxygen species, two more mechanisms that seem to complete the nephrotoxic effect [75].

Unlike CDDP, carboplatin allows for individualized dosing and easier administration. Although it presents different dose-limiting side effects, like myelosuppression and neurotoxicity, it faces the same platinating agent resistance in tumours, probably due to copper transporters like CTR-1, ATP7A, and ATP7B [83]. Copper transporters are also linked indirectly with the antioxidant defence inhibition and apoptosis induction [99]. The half-life of disappearance for carboplatin from plasma ranges from 33 to 49 hours with values higher than those of other platinum compounds but the pharmacokinetics of carboplatin are not influenced by the cumulative effect [102]. The preferred route of administration for carboplatin is intravenous or intraperitoneal for ovarian cancers, while the oral route is not so much used.

Prevention. Treatment. Prognosis

Several strategies have attempted to prevent or reduce cisplatin nephrotoxicity. Early experience suggested that the administration of cisplatin by prolonged continuous infusion associated with saline hyper-hydration, with or without furosemide or mannitol, reduces nephrotoxicity [20]. If applicable, the administration of lower doses of cisplatin in comparison with prior therapies, and concomitant hydration with intravenous isotonic saline should be introduced. Discontinuation of cisplatin is generally indicated in patients who develop evidence of progressive renal impairment. It has been demonstrated that hydration reduces the risk of nephrotoxicity in case high-dose carboplatin is used, however, it's apparently unnecessary with standard doses of 400 - 600 mg/m^2 [81].

The consequences of tubular toxicity that results in hypomagnesemia may be reduced by prophylactic administration of intravenous magnesium, to prevent or reduce the frequency and severity of tetany, convulsions or cardiac arrhythmias [7, 51].

Guidelines from the American Society of Clinical Oncology suggest that amifostine may be taken into consideration for protection against cisplatin nephro-toxicity although its use may be accompanied by side effects, including nausea, vomiting, flushing and infusion-related hypotension [41]. Other pharmacological agents that may ameliorate the nephrotoxicity due to their potential of reaction with nephrotoxic cisplatin metabolites to form less toxic products are: sulphur-containing compounds (e.g. sodium thio-sulfate), DDTC (sodium diethyldithio-carbamate), mesna, biotin, cephalexin and sulfathiazole [81]. Other protective mechanisms are derived from the blockade of possible mediators of renal vasoconstriction (e.g. aminophylline which inhibits adenosine) and BN-52063 (antagonize platelet-activating factor) [23] and inhibition of cisplatin's metabolism by procainamide [26].

Some antioxidants like capsaicin, glutamine, melatonin, N-acetylcysteine and selenium have shown a kidney protective role in animal studies [86]. Theophylline has also been assessed for its protective properties [65, 66], however the effectiveness has been only partially proven [10, 27, 33 ,69]. Different platinum analogues (e.g. nedaplatin, ormaplatin, oxaliplatin and zeniplatin) or compounds that could form a complex with cisplatin (e.g., alginates, methionine and procaine hydrochloride) have been developed to reduce cisplatin nephrotoxicity [86]. Liposomal and micro-sphere preparations have been designed to improve the therapeutic index of platinum compounds [50].

Treatment includes general supportive care for AKI and magnesium supplementation in the case of hypomagnesaemia [27]. For patients for whom cisplatin is contraindicated or for whom there is an increased risk of nephrotoxicity, the substitution of carboplatin with cisplatin can be considered [65].

Glomerular impairment, but not hypomagnesaemia, may improve partially in time [14, 92]. A longitudinal study involving 27 children concluded that there was no evidence of cisplatin recovery nephrotoxicity (measured by GFR and serum magnesium) over 10 years follow-up [93].

Antimetabolites

Methotrexate (MTX) has a wide range of use due to its anti-proliferative and immunomodulatory effects [88]. In paediatric oncology it represents an effective treatment for acute lymphoblastic leukaemia [58].

High dose regimens (over 500 mg/m^2) are susceptible to result in AKI, with an overall incidence between 2 - 12% [44] and is mainly due to precipitation of MTX and its metabolite in the distal tubular leading to tubular obstruction, decreased GFR, ARF and tubular cell death [78]. Similarly to cisplatin and IFO mechanisms for nephrotoxicity, MTX is also thought to produce direct tubular injury with necrosis *via* ROS modulation [57, 106].

A transient decrease in GFR has been explained by a MTX induced afferent arteriolar constriction effect, that results in reduced glomerular capillary surface area and diminished glomerular capillary perfusion. MTX induced AKI is reversible and non-oliguric, with a serum creatinine peak within the first week [39].

A major concern with its use refers to the situation when a decreased GFR impact the MTX urinary clearance and serum concentrations. 90% of MTX is excreted renally and therefore a previously decreased GFR diminishes the capacity to excrete MTX, resulting in toxic systemic concentrations. If the transient effects are prolonged, the risk of organ damage increases [57, 77]. MTX can also be responsible for SIADH and high MTX concentrations reduce folate concentrations within normal cells resulting in toxicity. Typical renal histology findings include glomerulosclerosis, tubular loss and interstitial fibrosis [92]. Risk factors include hydration status and urinary pH, with those who are dehydrated or have an acidic pH being at greater risk. Unilateral nephrectomy and concomitant use of other reno-toxic drugs are additional risk factors [77]. The methotrexate nephrotoxicity may be increased by genetic polymorphisms involved in folate metabolism [101].

With respect to prevention and treatment strategies, AKI caused by MTX can be reduced, although not completely mitigated, with hydration and urine alkalinisation to a pH above 7.0 (in order to increase its solubility) [65, 92]. Currently, high dose leucovorin is administered 24 - 36 h after MTX, with the purpose of saving normal tissues from MTX toxicity by reloading folate concentrations [105]. Glucarpidase has also been used in patients with elevated MTX plasma concentrations, having a cleavage effect of

MTX in two non-toxic metabolites and thus quickly decreasing its concentration, however it had no effect on intracellular concentrations of MTX [2, 44].

Pharmacokinetics and toxicology

As a folate analogue, MTX travels to non-fatty tissues of the body rapidly after administration reaching the kidney, the liver, the skin and the muscles. The hydroxylation of MTX reaches 50% in approximately 12 h after administration if no liver damage is present. Elimination of MTX is through renal excretion, the creatinine clearance being positively correlated with MTX clearance, decreasing only in association with ifosfamide and cisplatin therapy.

In recent studies, the administration of methotrexate through oral or subcutaneous route did not show any significant differences in plasma concentrations even if the patients received daily folate supplements to diminish the possible side-effects, folate supplements that should have acted competitively but did not [55]. However, prior cisplatin therapy or the patients age may influence MTX pharmacokinetics. Higher concentrations have been found in children aged over 12 as compared to those under this age and in patients with cisplatin associated therapy [21]. In high doses, MTX monitoring is imperative for the possibility of delayed elimination or toxicity (systemic toxicity, nephrotoxicity) [66].

Clofarabine, a purine nucleoside analogue, is approved for the treatment of refractory paediatric acute lymphoblastic leukaemia. Current studies have revealed various degree of nephrotoxicity, mostly AKI, ranging from 10 to 36%. The mechanism of nephrotoxicity is not completely understood [65].

Pharmacokinetics and toxicology

Clofarabine inhibits DNA polymerase and ribo-nucleotide reductase, inducing apoptosis in abnormal cell lines. Although it shows dermatological toxicity, gastrointestinal toxicity, CNS toxicity, haematological toxicity and hepatotoxicity, myelosuppression is the dose-limiting factor. The elimination is performed through renal excretion and it reaches half-life in approximately 5 h with a decrease in elimination 6 h after administration. Although the elimination is mainly through the kidney, other unknown mechanisms seem to be involved [53].

The nephrotoxicity in patients being treated with clofarabine is expressed by the presence of acute kidney injury after 15 days of exposure, and 55% percent of the treated patients in one study have presented this side-effect [80]. Data suggests that hepatotoxicity is directly correlated to the concentration of clofarabine (reaching 8 times higher concentrations in liver than the plasma levels in rats) [15] and similarly, by analysis of LD50 in mice, extensive data was obtained sustaining this finding at the renal level as well, with proof of toxicity over nerves and spleen [63].

Nitrosoureas

Prolonged therapy with nitrosoureas BCNU (carmustine), CCNU (lomustine), methyl-CCNU (semustine) and streptozocincan cause a slowly progressive, chronic interstitial nephritisthat which is generally irreversible [69]. Streptozotocin use is known to results in mild ARF, mild proteinuria and/or tubular toxicity, manifested by aminoaciduria, phosphaturia, uricosuria, glycosuria, bicarbonaturia or Fanconi syndrome [39, 52, 92].

The exact mechanism of nephrotoxicity is not completely elucidated. Alkylation of tubular cell proteins has been proposed.

Other cytotoxic drugs

Some other cytotoxic drugs commonly used in children have occasionally been associated with the development of nephrotoxicity.

Anti□tumour antibiotic actinomycin D has almost no nephrotoxic effects. However, it has been suggested that it may sensitize renal tissue to chronic damage from radiation doses that normally are not supposed to induce a future nephropathy [92]. Thus, in patients with Wilms tumour it might reduce the degree of compensatory renal hypertrophy [64].

Anthracycline Bardi *et al*. have described signs of proximal tubular toxicity (e.g. increased urinary excretion of N-acetylglucosaminidase) in 26 children receiving anthracycline treatment [8].

5-azacytidine has been reported to cause mild glomerular and extensive tubular toxicity resulting in Fanconi syndrome, which although usually reversible, may sometimes be extremely severe, leading to death [79].

Mithramycin is one of the therapeutic agents used mostly in children and adults solid tumour or Ewing Sarcoma, being one of the most common nephrotoxic drugs [48]. It is known to produce acute and chronic glomerular injury [44].

Anticancer drug monitoring by liquid chromatography-mass spectrometry

In contrast to the immunoassays which are based on the interaction between an antibody and a part of the molecule which is quantified and which could be compromised by cross-reactivity with molecules having similar structure, liquid chromatography-mass spectrometry is based on the analysis of the retention time of the molecule after separation on a chromatographic column and of the m/z values of the entire molecule and of one or more fragments (daughter ions) resulted by induced dissociation in the mass spectrometer of the molecule which is quantified (parent ion). In the case of complex biological matrices such as plasma, serum, urine etc. the removal of molecules that may contaminate the column is desired. The extraction techniques which have been developed for the small molecules of the drugs that are monitored in the biological

matrices are protein precipitation, solid phase extraction, liquid-liquid extraction, phospholipid removal. An internal standard is added to the calibration samples and to the sample having an unknown content of analyte prior to the beginning of the sample preparation in order to have the possibility of measuring the losses that occur during all the steps of the analysis [43].

The author Canal-Raffin M and her collaborators reported very sensitive methods for the quantitative determination of the two structural isomers cyclophosphamide and ifosfamide and of the methotrexate [16]. In the case of the method developed for the quantitation of cyclophosphamide and ifosfamide, a deuterated internal standard was employed (cyclophosphamide-d4). The extraction of the analytes and internal standard was carried out using dichloromethane. The extraction of the methotrexate and of the deuterated internal standard methotrexate-d3 was performed according to a solid phase extraction method that employed the Isolute® HAX column which contains a mixed sorbent non-polar (C8) and quaternary amine (-NR3+) for the isolation of acidic molecules from complex matrices such as urine and plasma. For mass spectrometric analysis the authors used a triple quadrupole mass spectrometer, Quattro-micro, produced by Waters, USA. The chromatographic separation of cyclophosphamide and ifosfamide allowed the elution of the two isomers at the retention time 6.2 minutes and 5.6 minutes respectively. The limit of detection (LOD) was 10 pg/mL and the lower limit of quantification (LOQ) was 20 pg/mL for all three anticancer drugs.

Conclusions

The potential severity, frequency, and unpredictability of the nephrotoxic treatment in childhood cancer make this subject as tremendously important. The mechanisms of nephrotoxicity derived from such treatment are not entirely elucidated and further studies into the safety of cancer treatment protocols are needed. Periodic renal monitoring should be instituted immediately after the treatment and within a long term follow-up.

Nowadays, the incidence of most paediatric tumours has increased, cancer being the leading cause of death in the US. In Europe, cancer rates (for most cancer types) have increased by 1-2% per year [99]. On the other hand, including the long term outcome of cancer in children, has been significantly improved in the last 10 years, and there is a significant decline in cancer mortality. This can be explained by improved diagnostic procedures and improved addressability in resource poor settings. However, this is not an entirely satisfying explanation since the improved diagnostic methods remained fairly constant despite the increasing prevalence of cancer [46, 95]. Therefore, further efforts must be made to understand this pattern of childhood cancer incidence and to identify more effective treatments with fewer nephrotoxic effects.

Even with a decreased mortality, the associated co-morbidities derived from nephrotoxicity are serious enough to consider an optimistic prognosis for the cancer as being unlikely. Further research is needed to provide less nephrotoxic drugs, to identify better reno-protective strategies and stratify the risk of nephrotoxicity. When evaluating the risk of different degrees of nephrotoxicity, efforts should be made to provide as many statistical relevant correlations, in order to periodically update the guidelines for cancer management. Thus, encouraging families to include their children in risk assessment clinical trials, of course, with ethical safeguards, would provide a huge benefit for paediatric oncology. Furthermore, collaborative research with shared benefits among partners from different countries and regions in the world would improve both the understanding of particular differences in pathophysiology and the potential outcomes.

The clinical management of paediatric cancers will always have the burden of the potential lack of adherence to chemotherapeutic protocols for children and their families. Thus, for the best outcome, a better understanding of adolescent patients and their families' values, even more detailed then in adults' oncology is needed. Management team will keep into account some of the age psychological features addressing their fears and need of compassion. For instance, some of the most dramatic nephrotoxic adverse effects are the seizures secondary to hypo-magnesaemia associated to platinum treatment. An inadequate prevention, or potential misdiagnose of the aetiology, a delay in treatment or lack of family support may negatively affect the future compliance and may lead to upraise of psychological troubles.

There are no extensive researches over the impaired quality of life related to nephrotoxic effects of cancer treatment, particularly those associated to progression of CKD towards ESRD. Thus, delving deeper into the social integration of such children and also the ethical issues related to decision making is paramount [68]. In order to carry out such research one would need involve a multidisciplinary team (patient, doctors, family, psychologist, other therapists, clergy) in order to assess the social and the cultural patterns that are indispensable for a good communication and for alleviating or diminishing the suffering caused [34, 35].

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