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ORIGINAL PAPER

Renal cell apoptosis induced by nephrotoxic drugs: cellular and molecular mechanisms and potential approaches to modulation

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Abstract Apoptosis plays a central role not only in the physiological processes of kidney growth and remodeling, but also in various human renal diseases and drug-induced nephrotoxicity. We present in a synthetic fashion the main molecular and cellular pathways leading to drug-induced apoptosis in kidney and the mechanisms regulating it. We illustrate them using three main nephrotoxic drugs (cisplatin, gentamicin, and cyclosporine A). We discuss the main regulators and effectors that have emerged as key targets for the design of therapeutic strategies. Novel approaches using gene therapy, antisense strategies, recombinant proteins, or compounds obtained from both classical organic and combinatorial chemistry are examined. Finally, key issues that need to be addressed for the success of apoptosis-based therapies are underlined.

Keywords Renal apoptosis · Nephrotoxic drugs

Abbreviations

AIF	Apoptosis induced factor
Akt/PKB	Serine/threonine kinase/protein kinase B
ATP	Adenosine triphosphate
Bid	Bcl-2 interacting domain
BIP	Bax inhibiting peptide
Cdk2	Cyclin-dependent kinase 2
c-FLIP	FLICE-like inhibitory protein
DIABLO	Direct IAP binding protein with low pI
DR 4/5	Death receptor 4/5
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ER	Endoplasmic reticulum
ERK	Extracellular signal-regulated kinase
ESRD	End-stage renal disease
FasL	Fas ligand
FLICE	Fas-associated-death domain like IL-1β-converting enzyme
GADD	Growth arrest and DNA damage-inductible
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
G-CSF	Granulocyte colony-stimulating factor
HEK	Human embryo kidney cells
HGF	Hepatocyte growth factor
HK-2	Human proximal tubular epithelial cell line
HSP70	Heat shock protein 70
IAP	Inhibitors of apoptosis proteins
IGF-1	Insulin-like growth factor 1
IRE-1α	Inositol-requiring enzyme 1alpha
JNKs	Janus kinases

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LLC-PK1	Lilly laboratories, culture-pig kidney type1 cells
MAPKs	Mitogen-activated protein kinases
MCT	Murine cortical tubular cells
MDCK	Madin-darby canine kidney cells
MDR	Multi drug resistance
MEK	MAPK kinase
NF-κB	Nuclear factor κB
NRK-52E	Normal rat kidney epithelia
OAT	Organic anion transporter
OCT	Organic cation transporter
Omi/HtrA2	High temperature requirement protein A2
PgP	P-glycoprotein
PI3K	Phosphatidylinositol-3-kinase
PIDD	p53-induced death domain
PLA2	Phospholipase A2
RAP	Receptor associated protein
RMIC	Renal medullary interstitial cells
ROS	Reactive oxygen species
RPT	Rat proximal tubular cells
RPTC	Rabbit proximal tubular cells
siRNA	Small interfering RNA
Smac	Second mitochondria-derived activator of caspase
TGF	Transforming growth factor
TNF	Tumor necrosis factor
TNFR	Tumor necrosis factor receptor
TRAIL	TNF-related apoptosis inducing ligand
TUNEL	Terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick-end labeling
VEGF	Vascular endothelial growth factor

Cells continuously receive survival or death signals from the local microenvironment [1]. In the kidney, death through apoptosis is a physiological process in nephrogenesis as well as in maintenance of tissue homeostasis. When developing as a response to drug exposure, apoptosis may, however, become a double-edged weapon. While it leads to tissue loss and organ dysfunction, it may also contribute to clear off intoxicated cells and to control compensatory proliferative responses. A large number of drugs are known to induce renal cell apoptosis in cell culture or in vivo (Table 1), and this is associated with renal dysfunction. In general, apoptosis occurs at low levels of drug exposure, whereas necrosis requires higher doses [2–4]. This makes the study of apoptosis particularly relevant for the clinical usage of drugs, since they are supposed to be used at doses and for durations of treatment that do not cause necrosis in the experimental animal. It must be emphasized that a small amount of detectable apoptosis in kidney epithelium can correspond to a large level of cell death, given the short half-life of apoptotic cells which are easily cleared and lost in the urine [5].

In this review, we present a general overview of the main cellular and molecular mechanisms of apoptosis observed in the kidney, describe three well characterized and clinically relevant examples of drug-induced renal cell apoptosis, and discuss the state of the art of apoptosis modulation in nephrotoxic kidney injury.

Molecular mechanisms and cellular pathways of apoptosis in the kidney

Generally speaking, there are two phases in apoptosis: a commitment and an execution stage [6–8]. Apoptosis can be initiated via two major pathways as illustrated in Fig. 1. The intrinsic pathway involves subcellular organelles such as mitochondria, lysosomes or endoplasmic reticulum, whereas the extrinsic pathway, also called death receptor pathway, involves the activation of death receptors in response to ligand binding. Both pathways lead to the activation of specific proteases called the executioner caspases (caspase-3 and -7), which results in the characteristic morphological signs of apoptosis that include membrane blebbing, cell shrinkage, and DNA fragmentation. Nephrotoxic drugs seem to act mainly through the intrinsic pathway, and it will be described in more detail (Fig. 2).

Intrinsic pathway

Specific sensors initiate this pathway and information is relayed from one organelle to another. Most signals triggered by nephrotoxic drugs eventually converge to the mitochondrial pathway [9] (Table 2). Mitochondrial injury leads to the release of caspase activators, such as cytochrome *c*, inhibitors of antiapoptotic responses such as Smac/DIABLO and Omi/HtrA2, and caspase-independent promoters of cell death such as Apoptosis-Inducing Factor (AIF), which is abundant in renal epithelium [10]. This process is under the tight control of several factors [11, 12]. Proteins of Bcl-2 family, which are either pro- or anti-apoptotic, function as "molecular integrators" for the mitochondrial pathway. Upon exposure to death signals, the pro-apoptotic proteins Bax and Bak undergo structural modifications [13] and alter the mitochondrial membrane integrity to cause the release of cytochrome *c* (anchored through cardiolipin at the outer surface of the mitochondria inner membrane [14, 15]) and the other pro-apoptotic molecules [16]. Bax is found all along the nephron but absent from the glomerulus [17]. Bax and Bak can be activated by BH3-only proteins (Bid, Bad, Bim, Bmf, Bik,

Table 1 Apoptosis induced by nephrotoxic drugs and selected nephrotoxicants: in vitro and in vivo models

Compound	In vitro		In vivo	
	Cell type	References	Species	References
Acetaminophen	Mouse PTC	[174]		
Adriamycin	Renal tubular cells	[175]	Rat	[176]
Aminoglycosides	MDCK	[95]	Rat	[94]
	LLC-PK1	[2]		
Amphotericin	LLC-PK1 / RMIC	[157]	Rat	[157]
Anaesthetic			Rat	[177]
Cadmium	WKPT	[178]		[178]
Cidofovir	Human tubules/HK-2	[144]		
Cisplatin	Mouse PTC	[71, 171, 180]		
	Mouse CDC	[181]		
Cyclosporine A	MDCK	[182]	Rat: subcortical and juxtamedullary kidney sections	[179]
			Tubular and interstitial cells	[107]
Dichloroacetic acid			Rat: proximal tubules	[183]
Diclofenac			Mice: proximal and distal tubular cells	[184]
3,4-Dideoxyglucosone-3-ene	Mouse PTC	[33]		
Doxorubicin			Rat: tubular epithelial cell and distal tubule cells	[175]
Endotoxins	Human tubular epithelial cells	[185, 186]	Mice (Fas–/–, TNFR1–/– TNFR2–/–)	[187]
			C3H/HeJ Mice	[188]
Fluoroquinolones			Human: distal tubular cells	[189]
Mercuric chloride	Cultured rat proximal tubular cells (WKPT)	[178]		
	LLC-PK1	[190]		
Microcystin			Rat: kidney cortex and medulla	[191]
Ochratoxin A	PRK	[192]		
	OK	[193]		
	NRK-52E	[193]		
Oxalate	MDCK	[194]		
Radio-contrast agents	MDCK	[195–197]		
	LLC-PK1	[197, 198]		
Rapamycin	Mouse PTC	[199]		
Statins	Cultured murine tubular cells	[200]		
Thiazides			Rat: distal convoluted tubular cells	[201]
Zoledronate			Human: tubular epithelial cells	[202]

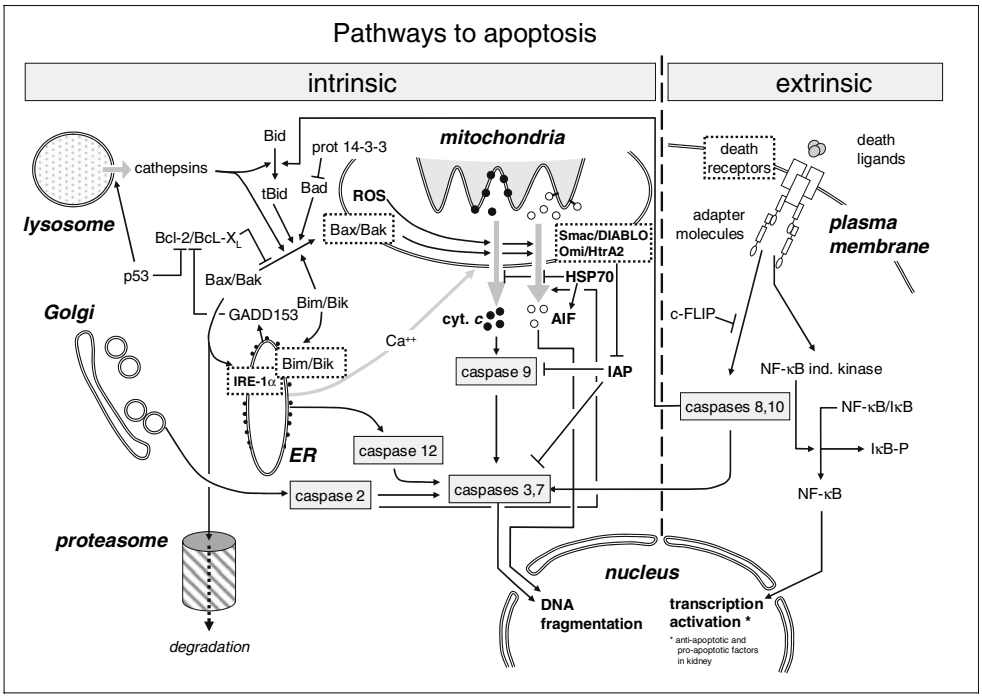

Fig. 1 Overview of the main pathways to apoptosis. Grey arrows denote the release of a factor from an organelle. Factors enclosed in dotted boxes are interacting with and/or inserted within the membrane of the corresponding organelle

Puma, Noxa and others [18]) behaving as sensors of cell stress. Reactive oxygen species (ROS) and caspases, especially caspase-2, may also lead to mitochondrial injury [19]. Once in the cytoplasm, cytochrome *c* interacts with an adaptor molecule, Apaf-1 and procaspase-9, forming the apoptosome that promotes the activation of caspase-9. Members of the inhibitor of apoptosis proteins family (IAPs) inhibit caspases and pro-caspases and, are themselves controlled by other mitochondrial proteins (Smac/DIABLO and Omi/HtrA2) [20]. Moreover, HSP 70 inhibits nuclear AIF accumulation by binding it in the cytosol [21], and reduces both AIF and cytochrome *c* release from the mitochondria through still incompletely established processes. Part of the cytoprotective effects of HSP70, as well as HSP27, is related to their ability to inhibit key effectors of the apoptotic machinery at the pre- and post-mitochondrial level [22]. In contrast, the antiapoptotic proteins Bcl-2 and Bcl-x_L bind to pro-apoptotic members of the Bcl-2 family, and impair the activation of Bax/Bak, thereby maintaining mitochondrial membrane integrity. In the adult human kidney, the expression of Bcl-2 is detected in the parietal epithelium of the Bowman's capsule and all along the nephron from the proximal tubules to the collecting ducts [23, 24]. The expression of Bcl-x_L is lower in S1 segment of the proximal tubules than in S3 segment and the distal tubules [25]. In parallel to the Bcl-2 family of proteins, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a pleiotropic enzyme overexpressed in apoptosis and in several human chronic pathologies, may also play a critical role in cytochrome *c* and AIF release [26].

The role of the proapoptotic Bcl-2-like proteins in drug-induced apoptosis has been well documented, such as for instance for gentamicin in LLC-PK1 cells [27], for cyclosporine A in MCT cells [28], and for cisplatin in animal models [29]. This role has been confirmed (i) by microinjection experiments with the central domain of Bax (Bax-syn) or of Bak (Bak-syn) [30], (ii) by antagonizing Bax with inhibiting peptides such as BIP (Bax Inhibiting Peptide, derived from Ku 70 [31][1]), (iii) by preventing the synthesis of Bax with antisense oligodeoxynucleotides [28, 33], or (iv) in Bax-deficient mice [29]. The impairment of Bax or Bik degradation by inhibition of the ubiquitin-proteasome pathway also results in increased apoptosis in cultured renal and extrarenal cells [27, 34]. In this context, bortezomib, a dipeptidyl boronic acid, which inhibits the 26S unit of the proteasome, induces rapid accumulation of Bik [35] and stabilizes Bak and Bax [36, 37] and causes apoptosis. Because both antiapoptotic and proapoptotic proteins may be degraded through the ubiquitin-proteasome pathway, this approach is challenging, especially since inhibition of proteasome is cell and stimulus-specific.

Whereas mitochondria represent the keystone of the apoptosis intrinsic pathway, other organelles may play a critical role upstream of mitochondria (Table 2). Cathepsins may be released from lysosomes into the cytosol and

[1] Although this paper has been formally retracted based on the discovery of image manipulations to improve the appearance of the figures, the binding of Ku70 and of its penta-peptide derivative to Bax, inhibiting its activation and Bax-induced cell death, have been confirmed[32].

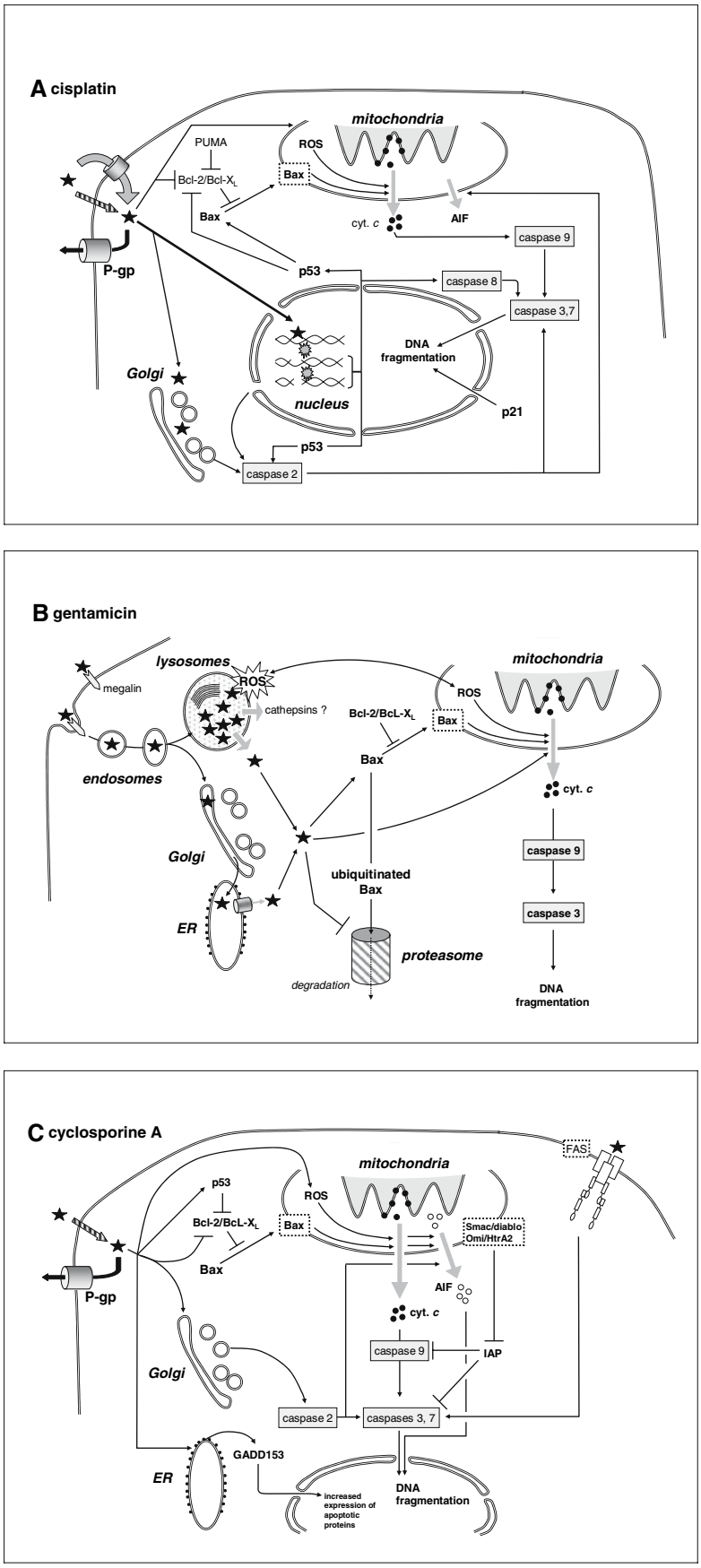

Fig. 2 Main mechanisms of apoptosis observed with three typical nephrotoxic drugs: upper panel (**a**), cisplatin; middle panel (**b**), gentamicin; lower panel (**c**), cyclosporine A. The drug is symbolized by a black filled star in each case

Table 2 Subcellular and molecular pathways involved in drug-induced apoptosis in the renal cells

Primary subcellular organelle	Drug	Evidences	Models	References
Mitochondria	Adriamycin	Release of cytochrome *c*	Rat proximal tubular cells	[203]
		Activation of caspase-9		
	Cisplatin	Release of Omi from mitochondria to the cytoplasm and degradation of XIAP	Primary mouse proximal tubule cells	[149]
		Effect of mitochondrial blockade	Renal collecting duct-derived cells (MDCK-C7)	[204]
		Decrease in relative ratio of Bcl-2/Bax in the outer medulla	Rat kidney	[205]
		Release of cytochrome *c*	Primary cultured rabbit proximal tubules	[206]
		Mitochondrial permeability transition	Murine renal proximal tubular epithelial	[207]
		Activation of Bax	LLC-PK1	[208]
		Mitochondrial permeability pore transition		
		Release of cytochrome *c*		
		Activation of caspase-9		
		Overexpression of MnSOD	Human embryonic kidney 293 (HEK293)	[209]
		Bad phosphorylation	LLC-PK1	[73]
		Activation of caspase-9		
		Translocation of endogenous Bax from the cytosolic to the membrane fractions	Mouse collecting duct cells	[3]
		Release of cytochrome *c*		
		Inhibition of complexes I to IV of the respiratory chain	LLC-PK1	[210]
		Inhibition of Na^+/K^+ ATPase	Renal proximal tubular cells	[66]
		Collapse of the mitochondrial membrane potential		
	Contrast agents	Decrease in relative ratio of Bcl-2/Bax	SHR Rats	[211]
	Cyclosporine A	Decrease in relative ratio of Bcl-2/Bax	Male Sprague–Dawley rats	[109]
		Translocation of Bax to the mitochondria	Murine proximal tubular epithelial cells	[28]
		Release of cytochrome *c* and Smac/DIABLO		
		Loss of mitochondrial membrane potential		
		Activation of caspase-9		
	Gentamicin	Loss of mitochondrial membrane potential	LLC-PK1	[2]
		Activation of caspase-9		
		Release of cytochrome *c*		
	G418	Release of cytochrome *c*	Rat kidney cells	[212]
	Statins	Decrease of Bcl-xL/Bax ratio	Murine tubular cells	[200]
		Release of cytochrome *c*		
		Activation of caspase-9		

Table 2 continued

Primary subcellular organelle	Drug	Evidences	Models	References
Endoplasmic reticulum	Acetaminophen	Upregulated expression of GADD 153	MCT cells (cultured line of proximal tubular epithelial cells harvested originally from the renal cortex of SJL mice)	[174]
		Caspase-12 cleavage		
	Cisplatin	Increased the activity of ER-iPLA(2)	Rabbit renal proximal tubule cells (RPTC)	[213]
	Cyclosporine A	Induction of GADD 153 expression	Murine proximal tubular epithelial	[28]
	G418	Increase in Ca^{2+} concentration	Rat kidney cells	[212]
		Cleavage of m-calpain and procasase-12		
	Tunicamycin	Decrease of apoptosis in renal proximal tubular epithelium in GADD 153−/− Mice	GADD 153−/− Mice	[45]
Lysosomes	Gentamicin	Release of acridine orange	LLC-PK1	[27]
		Lysosomal permeabilization		
	Iodinated contrast media (iobitridol and iohexol)	Prominent lysosomal alteration of the proximal convoluted tubular cells	Rats	[214]

can activate cytosolic pro-apoptotic proteins such as Bid and Bax [38]. The release of cathepsins has been observed upon activation of the p53 pathway [39] and may contribute to cell death induced by chemotherapeutic drugs [40]. The endoplasmic reticulum can initiate apoptosis directly or through cross-talk with mitochondria or lysosomes. A number of endoplasmic reticulum-specific proteins involved in drug-induced apoptosis have been described, such as caspase-12 and IRE-1α [41]. There is cross-talk between the endoplasmic reticulum and mitochondria [42]. Thus, translocation of Bim to the endoplasmic reticulum may lead to caspase-12 activation whereas Bax and Bak formed a protein complex with the cytosolic domain of IRE-1α essential for its activation and providing a physical link between members of the core apoptotic pathway and the unfolded protein response [43]. But signals from the endoplasmic reticulum may also activate the mitochondrial pathway. By targeting the membrane of the endoplasmic reticulum, Bik can initiate a release of Ca^{2+} which will trigger the release of cytochrome *c* to the cytosol [44]. Growth-arrest- and DNA-damage-inducible gene 153 (GADD153) is a transcription factor that is expressed upon endoplasmic reticulum stress and downregulates Bcl2 expression [45]. Endoplasmic reticulum and lysosomes may also interact. Siomycin A causes both lysosomal membrane permeabilization and endoplasmic stress [46]. The Golgi complex may also play a critical in the development of apoptosis through stack dispersion and disassembling into tubulo-vesicular clusters [47] and release of caspase-2. In this way, apoptosis can be initiated upon stress from the secretory pathway independently from mitochondria [48] or in a dependent way [19].

Extrinsic (receptor-mediated) pathway

Proapoptotic signaling can be triggered through the binding of death ligands (such as TNF [Tumor Necrosis Factor], Fas Ligand (FasL), TRAIL [Tumor necrosis factor-related apoptosis inducing ligand]) to their corresponding receptors (TNFR, Fas, DR4/5), through receptor trimerization, recruitment of adaptor proteins and activation of the initiator caspases 8 and 10. Activated caspase-8 then proteolytically activates caspase-3 and may recruit the mitochondrial pathway through the cleavage of Bid [1]. TNF and FasL are known to induce apoptosis in stressed tubular epithelial cells [49] and may be present in the kidney following nephrotoxic insults. Cellular FLICE [Fas-associated death-domain-like IL-1beta-converting enzyme]-inhibitory proteins (called c-FLIP) prevents the activation of procaspase-8 and, thereby, protects against death receptor-mediated apoptosis [50].

Caspase-dependent and -independent processes

While most nephrotoxic drugs induce caspase-mediated apoptosis, evidence is mounting for the existence of caspase-independent pathways, probably as a safeguard mechanism if caspase-mediated routes would fail [7]. A typical example of a nephrotoxic drug causing caspase-independent apoptosis is acetaminophen which can induce apoptosis even in the presence of caspase inhibitors [51]. The main caspase-independent pathway could be triggered by the release of AIF (which may act only when caspases are inhibited or not activated [52]), as demonstrated in cadmium-induced apoptosis in human embryonic kidney cells [53].

Modulators of the apoptotic pathway

In addition to downstream regulators like Bcl-2 family proteins, three important mechanisms have been reported to regulate cell survival. These are adhesion factors (integrins [54, 55], signal transducers such as protein kinases, and transcription factors such as NF-kB and p53 [56].

Mitogen-Activated Protein Kinases (MAPK) are upstream modulators of apoptosis. They play an important role in the toxic injury induced by puromycin [57]. There are three MAPK pathways. The ERK pathway generally inhibits apoptosis while the JNK (predominantly detected in the adult kidney [58]) and p38 kinase pathways, promote apoptosis. Two other kinases, PI3K and Akt/PKB, also act as modulators of the default pathway. The activated form of Akt/PKB phosphorylates Bad, which then associates with the chaperone protein 14-3-3 and becomes unable to exert its pro-apoptotic function [59]. NF-kB is sequestered in the cytosol by inhibitory proteins known collectively as IkB. A wide range of apoptotic triggers cause the proteasomal degradation of I-kB [60], allowing the active NF-kB to translocate to the nucleus. This results in an increased transcription of a large number of proteins involved in inflammation, apoptosis and cell proliferation. While the net effect of NF-kB is usually anti-apoptotic [61], NF-kB activation in kidney can also lead to stimulation of apoptosis in renal cells. This is exemplified by TRAIL-mediated NF-kB activation, which increases DR5 [death receptor 5] expression, and amplifies the apoptotic response of TRAIL in kidney derived epithelial cells [62].

p53 is a tumor suppressor mutated in many forms of neoplasia and known as the "the guardian of the genome" [63]. p53 stimulates apoptosis by promoting expression of genes that encode apoptotic proteins. However, it also has transcriptionally independent activities. These functions involve a direct interaction of p53 with members of the Bcl2 family of proteins, allowing p53 to function as a BH3-only protein [64].

Typical examples of apoptosis induced by nephrotoxic agents

Among the many agents listed in Table 1, we selected three nephrotoxic agents (cisplatin, gentamicin, and cyclosporine A) based on their illustrative behaviors of specific pathways to apoptosis (Fig. 2) and their clinical importance in major therapeutic areas, namely cancer, infectious diseases and immunosuppression.

Cisplatin or the "p53-mitochondria" cross-talk

Cisplatin blocks DNA replication and gene transcription by inducing single and double-strand DNA breaks. Nephrotoxicity is a limiting factor for its use as anticancer agent [65] and is related to its accumulation in kidney [66] via both passive [67] and active transport [68–70]. Cisplatin is also subject to MDR1/P-glycoprotein efflux [69]. Cisplatin nephrotoxicity is characterized by a reduced renal perfusion and a concentrating defect [70, 71]. Cisplatin injures essentially the S1 and S3 portions of the proximal tubules and the distal tubules [25, 66, 70, 72]. The nephrotoxic potential of cisplatin is multifactorial and includes inflammatory reactions and induction of tubular cell apoptosis. Cisplatin recruits the Bax-mediated mitochondrial pathway for apoptosis and activates initiator caspases-8, –9 and –2, and executioner caspase-3 in cultured tubular cells and in vivo [29, 73]. Increased expression of p53 appears critical for apoptosis induction [74–76]. Normal kidney epithelium expresses high p53 levels [77]. Available evidence points to a role of the transcriptional activity of p53 in nephrotoxicity. PUMA and p53-induced death domain protein (PIDD) are critical p53 targets, the expression of which is induced by cisplatin. PUMA antagonizes Bcl-x_L via molecular interaction [78]. PIDD promotes the activation of caspase-2, which causes the release of AIF [79]. Inhibition of p53, caspase-2 or AIF markedly protected from cisplatin induced apoptosis in cultured tubular cells [79]. Although less characterized in the particular case of cisplatin, p53 may also have non-transcriptional actions inactivating Bcl2/Bclx$_L$ and activating Bax [80]. In addition, cisplatin activates the MAPKs ERK, JNK, and p38, both in vivo and in vitro [71, 73]. In the context of cisplatin nephrotoxicity ERK promotes apoptosis, contrary to its usual role in cell death regulation [71]. p38 has no direct effect of apoptosis induction in cultured cells [71], but its inhibition had a beneficial effect in vivo through decreased TNF production [81]. Cisplatin also decreases Bcl-x_L [78], increases oxygen radical production [82] and increases Cdk2 activity, which, in turn, recruits E2F1, a key regulator that links cell cycle progression and cell death, both in vitro and in vivo [83–85].

Cdk2 and E2F1 are key mediators of cisplatin induced apoptosis in nephrotoxicity. Cisplatin also activates survival pathways, but these may not be sufficient to allow survival of many cells. Thus, cisplatin activates Akt/PKB [73] and increases the expression of p21 cyclin-dependent kinase (Cdk) inhibitor, which inhibits Cdk2 [84]. Studies are under way of structure-activity relationships with new platinum derivatives with unusual selectivity and less toxicity [86].

Gentamicin or the "lysosome-mitochondria" cross-talk

Clinical nephrotoxicity induced by gentamicin (the most studied molecule in this family of the aminoglycoside antibiotics) manifests itself clinically as non-oliguric renal failure with a slow rise in serum creatinine and a defective urinary concentrating ability developing after several days of treatment. These changes are preceded and accompanied by signs of tubular dysfunction (release of brush-border and lysosomal enzymes, renal wasting of K^+, Mg^{2+}, Ca^{2+} and glucose) [87]. After glomerular filtration, a small but significant proportion of the administered dose of gentamicin is retained in the epithelial cells lining the S1 and S2 segments of the proximal tubules [88, 89]. The drug enters cells by adsorptive/receptor mediated endocytosis after binding to acidic phospholipids and megalin [90, 91], and is found essentially in lysosomes [92, 93]. Animals treated with low, therapeutically relevant doses of aminoglycosides show both lysosomal phospholipidosis and apoptosis in proximal tubular cells [94]. Apoptosis induced by aminoglycosides has been reproduced in vitro with LLC-PK1 and MDCK cells and found to be directly related to the amount of drug accumulated by the cells [95].

Two, non-mutually exclusive, mechanisms have been proposed to link a cytosolic distribution of gentamicin and apoptosis. Cell culture studies, combined with the use of membrane models, show that gentamicin destabilizes the lysosomal membrane [2], which could result in release to the cytosol of the drug and lysosomal constituents such as cathepsins. In parallel, morphological studies using labeled gentamicin suggest a retrograde transport of endocytozed gentamicin through the Golgi complex and the endoplasmic reticulum from which it may be released to the cytosol [96].

Quite interestingly, gentamicin introduced directly in the cytosol by electroporation (thus bypassing the endocytic route) also induces apoptosis at very low concentrations [27]. This indicates that (a) only a small fraction of the amount of gentamicin stored in lysosomes (or transiting through the Golgi) needs to be released in the cytosol to trigger apoptosis; (b) it is probably the release of the drug itself, not of the lysosomal constituents, which is critical. In this context, the storage of gentamicin in lysosomes would actually appear as a protective mechanism rather than a toxic event, as long as the drug is prevented from moving from there to the cytosol.

The next steps appear rather straightforward, and involve mitochondrial activation with the release of cytochrome *c* and activation of caspase-3 [2], which can be prevented by overexpression of Bcl-2 [95]. Cytosolic gentamicin could act directly on mitochondria (polycations are known to induce the release of soluble intermembrane proteins from mitochondria, in vitro [97]) or indirectly through impairment of Bax proteosomal degradation, evidenced by an increase ubiquitinated Bax [27], since gentamicin binds to the β type 9 subunit of the proteasome [98]. Gentamicin triggers the generation of ROS in vitro [99] in the presence of polyunsaturated lipids, which could also participate to this process.

Cyclosporine A: focus on mitochondria

Cyclosporine A is a calcineurin inhibitor which revolutionized the control of graft rejection, the earliest and most notable successes being obtained in kidney transplantation. Ironically but sadly enough, its use quickly appeared limited by nephrotoxicity [100, 101]. Chronic cyclosporine A nephrotoxicity, characterized by tubular atrophy and interstitial fibrosis with progressive renal impairment, contributes to chronic kidney allograft nephropathy (the main cause of graft loss after 1 year), and is a risk factor for the occurrence of end-stage renal disease (ESRD), which is manageable but often requires chronic dialysis [102]. Renal tubular injury is a consequence of renal vasoconstriction and endothelial injury leading to ischemia, as well as a direct toxic effect of cyclosporine A on tubular epithelium [103]. Cyclosporine accumulates in renal tissue [104], but is also a substrate of P-glycoprotein [105], and a low P-gp expression in patients has been associated with increased occurrence of nephrotoxicity [106].

Apoptosis has been clearly evidenced in tubular and interstitial cells of transplanted patients with chronic cyclosporine nephrotoxicity [106]. Tubular cell apoptosis is also observed in animal [107–109] and cell culture models [110, 111]. Cyclosporine-induced apoptosis is primarily triggered through the mitochondrial pathway. The generation of ROS (indirectly demonstrated in vitro in tubular epithelial cells through the protective effect of prednisone [112]), the reduction of Bcl-2 and IAP expression, the increased expression of Bax (in mesangial cells [113] and well as in vivo [109]) and the translocation of Bax to the mitochondria (in murine tubular epithelial cells [28]) all contribute to apoptosis induction. While increased p53 expression has been observed in tubular cells exposed to cyclosporine in culture and in vivo [114, 115],

Table 3 Main approaches toward reduction of drug induced renal apoptosis

Drugs responsible for renal apoptosis	Protectant(s)	Model(s)	Effect observed	References
Decrease of the uptake or accumulation of the drug inducing apoptosis				
Competition with drug binding				
Aminoglycosides	Ca^{2+} (diet supplementation)	Rats	Decrease of blood urinary nitrogen	[215]
	Aminoglycosides	Rats	Decrease of renal cortical concentrations	[216]
	Ligands of megalin (lysozyme, aprotinin, cytochrome *c*, apolipoprotein E3) or chaperone proteins (RAP)	Rats	Reduction of renal cortical concentrations	[121]
		Mouse	Decrease of *N*-acetyl-glucosaminidase release from lysosomes	[122]
		LLC-PK1		
Impairment of transport				
Cidofovir	Probenecid inhibit the OAT1	Primary cultures of human proximal tubular cells	Prevention of apoptosis	[144]
Cisplatin	Up-regulation of transporters like MDR1 and P-gp and down-regulation of organic ion transporters OAT's	Rats	Increased gene expression	[69]
	Competition of uptake by hOCT2	HEK 293	Decrease of cell apoptosis	[119]
Cyclosporine A	Changes of chemico-physical properties of the membrane	LLC-PK1	Decrease of cell apoptosis	[123]
Hydrophobic compounds	Inhibition of carriers (e.g.L-FABP)	Rats		[217]
Increase of elimination				
Aminoglycosides	Raising the urine pH	Rats	Decrease of cortical drug accumulation	[218]
Cisplatin	Osmotic diuretics	Mice	Decrease of cell apoptosis (AnnexinV-FITC; TUNEL)	[124]
Modulation of pro- and anti-apoptotic proteins and/or pathways involved in apoptosis				
Inhibition of caspases				
Cisplatin	Pan caspase inhibitor Caspase-1 and -3 inhibitors	RPT	Decrease of cell apoptosis (Annexin V flow cytometry; in situ end labelling of fragmented DNA, light/electron microscopy; DNA laddering)	[143]
	Pan caspase inhibitor	LLC-PK1	Prevention of caspase activation and apoptosis	[219]
Cyclosporine A	Pancaspase inhibitor Caspases-3, -9 and -2 inhibitors	MCT	Prevention of apoptosis and increased long-term survival	[28]

Table 3 continued

Drugs responsible for renal apoptosis	Protectant(s)	Model(s)	Effect observed	References
Action on proteins of Bcl-2 family				
Cisplatin	Minocycline	Rat kidney proximal tubular cells	Upregulation of Bcl-2	[127]
			Suppression of Bax accumulation	
			Decrease of outer membrane damage	
			Inhibition of cytochrome *c* release	
	Overexpression of Bcl-2	Mouse collecting duct cells	Suppression of Bax translocation	[3]
	Molecules like Omi able to bind and cleave inhibitors of apoptosis proteins	Primary mouse proximal tubule cells	Upregulation of Omi protein	[149]
			Release of Omi from mitochondria to the cytoplasm	
			Degradation of XIAP	
Cyclosporine A	Bax antisense oligonucleotide	MCT	Decreased number of apoptotic cells (morphological studies)	[28]
Gentamicin	Bcl-2 Cell Transfection	LLC-PK1 and MDCK	No visible DNA laddering	[95]
Tacrolimus	Synthetic peptides derived from proteins of the Bcl-2 family	LLC-PK1	Decreased apoptosis (for Bcl-2-derived peptides)	[30]
			Pro-apoptotic effect for Bax- and Bak-derived peptides annexin V assay; morphology)	
Action on cellular pathways				
Cisplatin	MAPK/ERK kinase (MEK) inhibitor	Mice	Decreased number of apoptotic cells (morphological studies)	[150]
			Decreases caspase-3 activity	
	Carbon monoxide-releasing molecule acting as a guanylate cyclase activator	Rat	Decrease of apoptosis in tubules at the corticomedullary junction	[220]
	Prevention of the inhibition of PPAR-α activity	LLC-PK1	Decreased translocation of Bax	[221]
			Increase of Bcl-2 expression	
	Agonists of PPAR-γ (rosiglitazone)	Cultured human kidney	Decreased number of apoptotic cells (morphological studies)	[222]
	Nutlin-3 acting by activating p53 pathway	Rat kidney proximal tubular cells	Suppression of Bax/Bak activation	[75]
	Inhibitor of NF-κB like parthenolide	Male Wistar Rats	Decreased number of TUNEL-positive cells	[223]
Adriamycin	Prostacyclins as a suppressor of the activation and translocation of nuclear NF-κB	NRK-52E	Decreased caspase-3 and 9 activation	[203]
			Inhibition of cytochrome *c* release	
			Increase of Bcl-2 expression	

Table 3 continued

Drugs responsible for renal apoptosis	Protectant(s)	Model(s)	Effect observed	References
Ligands or proteins specific to receptor-mediated pathway				
Cisplatin	Genetic deletion of either TNF-alpha or TNFR2	RPT	Reduced cisplatin-induced renal failure, necrosis and apoptosis	[152]
Cyclosporine A	Mineralcorticoid receptor blockers	Rat: subcortical and juxtamedullarly cells	Decrease of TUNEL positive cells	[179]
Inhibition of oxidative stress				
Acetaminophen	IH636 grape seed proanthocyanidin extract	Mice	Decreased DNA fragmentation	[135]
			Prevention of renal apoptosis (histology)	
Adriamycin	Tetramethylpyrazine	NRK-52E cells	Decreased apoptosis	[176]
Cisplatin	Reduced glutathione	LLC-PK_1	Decreased DNA fragmentation	[224]
	Edaravone (hydroxy radical scavengers)	Murine proximal tubular cells	Reduced mitochondrial transmembrane potential loss	[207, 225]
		Male Wistar rats	Decreased number of TUNEL positive cells in cortical renal tubules	[225, 226]
	Dimethylthiourea (hydroxyl radical scavengers)	Adult male Wistar Rats	Prevention of the increase of caspase-3 activity	[227]
		Primary cultured rabbit proximal tubule	Decreased cytochrome *c* release from mitochondria	[206]
			Reduction of caspase-3 activation	
	Tiron (superoxide scavenger)	Primary cultured rabbit proximal tubule	Decreased cytochrome *c* release from mitochondria	[206]
			Reduction of caspase-3 activation	
	N-Acetylcysteine	LLC-PK1	Prevention of apoptosis	[219]
	Oxathiazolidine derivative (cystein-prodrug)	LLC-PK1	Prevention of apoptosis	[228]
	Trolox (anti-oxidant)	LLC-PK1	Prevention of apoptosis	[219]
	Inhibition of CYP2E1	Mice CYP2e1–/–	Prevention of renal apoptosis (histology)	[137]
	Sodium dependent glucose transporter (SGLT1) activator	LLC-PK1	Reduction of peroxynitrite production	[229]
	Manganese superoxide dismutase	Mn SOD transfected human embryonic kidney 293 cells	Prevention of renal apoptosis (Annexin V binding assay)	[209]
Cisplatin and gentamicin	Flavonoids found in Pongamia pinnata (kaempferol and 3,5,6,7,8-pentamethoxy flavone)	Rats	Prevention of renal apoptosis (histology)	[133]

Table 3 continued

Drugs responsible for renal apoptosis	Protectant(s)	Model(s)	Effect observed	References
Gentamicin	Superoxide dismutase and catalase	Rats	Decrease of gentamicin-induced mesangial cell apoptosis	[230]
	Tetramethylpyrazine	NRK-52E	Decrease in ROS formation	[134]
			Decrease of caspase-3/8 and -9 activities	
			Inhibition of increase in Bcl-x_L expression	
	Chelerythrine	Rats	Prevention of renal apoptosis as evidenced by histological studies	[231]
	Kallikrein/kinin	Rats	Protection of renal apoptosis	[232]
Radiocontrast Agents	Ascorbic acid	Humans	Prevention of renal injury	[233]
Enhancement of vascular effects				
Radiocontrast Agents	Hydration	Humans	Prophylactic regimen for radiocontrast therapy	[234]
Cyclosporine A	Blockage of angiotensin II receptors	Rats	Decrease in apoptosis in rats treated with cyclosporine A and losartan	[107]
Survival factors				
Amphotericin B	Epidermal growth factor (EGF)	Sprague–Dawley rats	Prevention of renal apoptosis in tubular epithelial cells and mesangial cells	[157]
Administration of TRAIL, a member of the death receptor ligand family		HEK 293	Protection from TRAIL-induced apoptosis in a dose-dependent manner	[235]
			Inhibition TRAIL-mediated cytochrome *c* release from the mitochondria and caspase-3-like activation	
Amphotericin B	Insulin-like growth Factor-1 (IGF-1)	LLC-PK1 medullary interstitial cells	Protection against apoptosis of renal proximal tubular cells	[157]
Cidofovir		Human proximal tubular epithelial cell line (HK-2)	Protection against apoptosis of renal proximal tubular cells	[144]
Cisplatin		Mouse inner medullary collecting duct cells	Protection against apoptosis of renal epithelial cells	[236]

Table 3 continued

Drugs responsible for renal apoptosis	Protectant(s)	Model(s)	Effect observed	References
Cidofovir	Hepatocyte growth factor (HGF)	Human proximal tubular epithelial cell line (HK-2)	Protection against apoptosis of renal proximal tubular cells	[144]
Cisplatin		Mice	Prevention of apoptosis of renal cells	[161]
		Mouse inner medullary collecting duct		[236]
Cyclosporine A		Rats transfected with HGF gene	Rescue of cyclosporine A-induced tubular injury	[159]
		Human proximal tubular cell	Inhibition of tubular cell apoptosis	
			Increase of the number of proliferating tubular epithelial cells	
			Reduction of apoptosis in glomerular epithelial cells	
Cyclosporine A	Anti-TGF-beta1 antibody	Male ICR mice	Decreased number of apoptotic cells in cortical tubular epithelium	[237]
Cisplatin	Antibodies to EGFR Pan-EGFR-inhibitor	Immortalized mouse proximal tubular cells	Prevention of renal apoptosis in tubular cells	[71]

no functional studies on its role in nephrotoxicity have been performed.

In cultured tubular cells, caspases-2, –9 and –3 are directly activated by cyclosporine [26]. The pan-caspase inhibitor zVAD does not prevent Bax translocation to mitochondria or cytochrome *c* release. By contrast, zVAD prevents the loss of mitochondrial membrane potential [28]. Thus, Bax causes cytochrome *c* release, which activates caspases which, in a positive feed-back loop, further damages the mitochondria and leads to loss of mitochondrial transmembrane potential.

In addition, there is also evidence of cyclosporine-induced endoplasmic reticulum stress, as witnessed by the increased expression of GADD153 [28]. However, caspase-12 was not processed, suggesting that the full endoplasmic reticulum stress response was not recruited by this drug, contrary to the effect of acetaminophen [169]. Cyclosporine increases Fas expression in tubular epithelium in culture and in vivo [28, 109, 116]. However, this appears to be an epiphenomenon which does not participate in apoptosis induction since cyclosporine does not sensitize FasL-induced apoptosis and does not increase caspase-8 activity [28].

Strategies to decrease apoptosis

Apoptosis regulators have emerged as key targets for the design of therapeutic strategies aimed at modulating cellular life-and-death decisions [117]. To be therapeutically meaningful, interventions at this level must take into account the fact that apoptosis, as outlined in the introduction, is a "double-edged weapon" being beneficial in many situations but also deleterious in others. Within the context of the loss of parenchymal cells caused by nephrotoxic drugs, inhibition of apoptosis, combined with the stimulation of the kidney regenerative processes, seems, however, clearly beneficial [118]. We will briefly discuss here the main strategies designed so far in this context (Table 3), but the reader must realize that none of them will be successful if the primary pharmacological activity of the drugs under study is not maintained to an acceptable level. Moreover, these strategies must include a targeting component to make them specific to the kidney tubular epithelial cells.

Decreasing drug accumulation in the kidney

Most nephrotoxic drugs are excreted by the kidneys and accumulate in tubular cells to a greater degree that in other cells, as a result of increased local drug concentration and the presence of cell-specific transporters. Renal cell

accumulation of the drugs frequently does not contribute to the therapeutic effect. As apoptosis is most often directly related to the accumulation of a nephrotoxic drug in target cells, reduction of its uptake seems the most rational approach. Thus, down-regulation or inhibition of renal drug transporters (e.g., OCT2 and OAT1) or receptors (e.g., megalin) has been successfully attempted for cisplatin [69, 119], cidofovir [120] or aminoglycosides [121, 122], and has been incorporated into current clinical practice in the case of cidofovir [120]. In a more indirect fashion, modulation of membrane fluidity could be attempted. Cilastatin prevents the cyclosporin-induced decrease in membrane fluidity, thus inhibiting its transport across membranes and reducing its access to mitochondria and apoptosis [123]. Stimulation of efflux transporters could also be useful, as low P-glycoprotein is associated with an increase toxicity of cyclosporine. The simple increase in diuresis to foster the rapid elimination of the nephrotoxic agents is also of common usage with cisplatin or cidofovir [124].

Modulation of major apoptosis pathways

Targeting the modulators of the intrinsic and the receptor-mediated pathways to apoptosis represents a promising approach.

Because of their central role in the mitochondrial pathway to apoptosis, pharmacological manipulation at the level of the Bcl-2 family proteins has been attempted to modulate cell death [125]. Thus, Bax antisense oligodeoxynucleotides have been shown to protect from cyclosporine A- and 3,4-dideoxyglucosone-3-ene (a major glucose degradation product) -induced apoptosis in MCT cells [33]. Administration of Ku-70-derived peptide, an antagonist of Bax, has similar effect [33, 126]. Conversely, in vivo up-regulation of Bcl-2 reduces the number of kidney epithelial cells entering in apoptosis after treatment with cisplatin [127]. In vitro overexpression of Bcl-2 prevents the apoptosis of MDCK and LLC-PK1 cells induced by gentamicin [95], and microinjection of the NH_2-terminal region of Bcl-2 (Bcl2-syn) protects LLC-PK1 cells against tacrolimus-induced apoptosis [30]. Increase of Bcl-x_L (combined with a decreased of Bax and p53 production) induced by dexamethasone protects against apoptosis induced by puromycin in podocytes [128]. But more specific approaches will be needed, which could be represented by gene silencing techniques based on small interfering RNA (siRNAs). These have already been developed for downregulating diverse proapoptotic genes in cultured tubular cells [129]. In addition, systemic delivery of decreased tubular expression of proapoptotic proteins in renal ischemia reperfusion injury in mice, demonstrates the feasibility of this approach in vivo [130, 131]. Recently the protective effect of the small molecule Nutlin-3 against cisplatin-induced apoptosis was shown to be dependent on the prevention of Bax and Bak oligomerization [75].

Scavenging ROS has also been a popular approach, given their effect on lysosomal and mitochondrial pathways to apoptosis. Various anti-oxidants have been successfully used to prevent gentamicin and cisplatin [66, 132–134], as well as acetaminophen toxicity [135]. Indirect strategies have involved the inhibition of cytochrome P_{450} 2E1, a labile isoform involved in free radical generation [136] considered as a source of iron in cisplatin-induced renal injury [137], or the chelation of iron in kidney cells [138].

Inhibition of death receptor signaling represents an additional approach to reduce apoptosis induced by nephrotoxic drugs, with significant results for cisplatin [139]. The protective effect exerted by pentoxifylline against cisplatin nephrotoxicity [140] and the lower ability to induce apoptosis for amphotericin B-arabinoglycan as compared to amphotericin B-deoxycholate [141] could result from inhibition of TNF-α production and ensuing dampening of death receptor signalling.

Inhibition of caspase-dependent processes

Direct caspase inhibition is currently under active investigation [8, 142], and proof-of-concept data have been obtained in several experimental models involving cisplatin [143], cyclosporine A [28], or cidofovir [144], leading to the emergence two dipeptidyl pan-caspase inhibitors (z-VD-fmk or MX-1013 [145] and 2,4-dichloro-cbz-VD-fmk or MX 1122 [146]) and an inhibitor of caspase-3 and –7 (IDN-8050 [147]). The existence of tubular cell specific transporters may be used to specifically target these inhibitors. Gene silencing approach has also been developed to block the expression of caspase-3 and caspase-8 in vivo in renal ischemia/reperfusion injury models [130, 131]. A major difficulty lies however, in the very large number of substrates of caspases (>280) which includes proteins with important roles in cell structure, signaling, transcription and intercellular adhesion [148].

Beyond targeting caspases with exogenous inhibitors, modulation of their endogenous regulators such as IAPs, c-FLIPs and Smac/DIABLO might also be attractive. This strategy, currently in development for treatment of diseases in which deregulation of the apoptotic cell death pathway has been implicated, may now receive more attention for drug-induced nephrotoxicity (e.g., inhibition of Omi/HtrA2 in cisplatin-induced apoptosis [149]). Likewise, the MEK inhibitor U0126 decreases caspase-3 induced apoptosis by cisplatin by impairing ERK1/2 phosphorylation and affords significant functional and histologic protection [150].

Survival growth factors and Cdk inhibition

The progressive unraveling the complex growth factor/cytokine network in the kidney [151–153] may allow for entirely novel strategies to prevent apoptosis induced by nephrotoxic drugs.

The most successful approaches have dealt so far with the administration of survival growth factors. Thus, exogenous EGF (constitutively expressed in the distal convoluted tubules and in the thick ascending limb of Henle [154]) accelerates renal tubular cell regeneration after exposure to nephrotoxic drugs [155]. Intriguingly enough, monoclonal antibodies to EGFR or pan-EGFR inhibitors have been shown to prevent cisplatin-induced apoptosis, perhaps because cisplatin activates EGFR in the kidney, leading to ERK activation, a prodeath process in this case [71]. Likewise, IGF-1, which in ischaemia/reperfusion injury has comparable effects on apoptosis as caspase inhibition [156], protects against apoptosis induced by amphotericin B in the kidney [157] and cidofovir in cultured cells [144]. HGF protects from renal ischemic injury [158] and has beneficial effects on cidofovir-induced apoptosis in vitro [144]. Electroporation-mediated HGF gene transfer inhibits tubular apoptosis induced by cyclosporine A in vivo [159]. The antiapoptotic signaling IGF-1, EGF and HGF is mediated by the PI 3-kinase/Akt/PKB pathway [156], probably converging at Bad phosphorylation [160]. Hematopoietic cytokines, such as G-CSF have also been successfully used to protect against cisplatin-induced acute renal injury in mice [161], and endogenous VEGF protects against cyclosporine A-induced tubular cell apoptosis in vivo and in cell culture [162].

Modulation of the cell cycle regulation may also be a promising approach. Cell cycle arrest at G1/S or G2/M phase, induced by cyclin B1 and cyclin D1 is indeed known to contribute to apoptosis. Yet, inhibiting Cdk2 activity decreased apoptosis in growth factor-deprived mesangial cells [163]. In this context, sodium arsenite, which down-regulates the expression of cyclins, has beneficial effects on cisplatin–induced acute renal failure [83], and the Cdk inhibitor roscovitine, recently used in vivo to prevent the progression of polycystic kidney disease [164], has been shown to protect cultured mouse kidney proximal tubular cells from cisplatin-induced apoptosis [165].

Concluding remarks

The intracellular components of the apoptosis cascade have now been largely unraveled, revealing specific cellular factors and pathways that can be used as targets and should enable us to design strategies aiming at controlling cell death responses. Proteomic and microarray analysis may soon provide us with more targets, as exemplified by what has been shown for gentamicin [166–168], cyclosporine A [114, 169], cisplatin [69, 167, 170–172], and cidofovir [173]. We, however, need still to better understand the crosstalks between different pathways, to control the cell-specificity of the interventions, and to define optimal therapeutic schemes. Patient's genetic background may also prove critical. While highly challenging, the approaches outlined in this review may allow bringing promising preclinical findings to actual therapeutic practice.

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References

1. Lemasters JJ (2005) Dying a thousand deaths: redundant pathways from different organelles to apoptosis and necrosis. Gastroenterology 129:351–360
2. Servais H, Van Der Smissen P, Thirion G et al (2005) Gentamicin-induced apoptosis in LLC-PK1 cells: involvement of lysosomes and mitochondria. Toxicol Appl Pharmacol 206:321–333
3. Lee RH, Song JM, Park MY, Kang SK, Kim YK, Jung JS (2001) Cisplatin-induced apoptosis by translocation of endogenous Bax in mouse collecting duct cells. Biochem Pharmacol 62:1013–1023
4. Raffray M, Cohen GM (1997) Apoptosis and necrosis in toxicology: a continuum or distinct modes of cell death? Pharmacol Ther 75:153–177
5. Hauser P, Oberbauer R (2002) Tubular apoptosis in the pathophysiology of renal disease. Wien Klin Wochenschr 114:671–677
6. Cummings J, Ward TH, Ranson M, Dive C (2004) Apoptosis pathway-targeted drugs–from the bench to the clinic. Biochim Biophys Acta 1705:53–66
7. Broker LE, Kruyt FA, Giaccone G (2005) Cell death independent of caspases: a review. Clin Cancer Res 11:3155–3162
8. Lavrik IN, Golks A, Krammer PH (2005) Caspases: pharmacological manipulation of cell death. J Clin Invest 115:2665–2672
9. Kim R, Emi M, Tanabe K (2005) Role of mitochondria as the gardens of cell death. Cancer Chemother Pharmacol 57:545–553
10. Daugas E, Nochy D, Ravagnan L et al (2000) Apoptosis-inducing factor (AIF): a ubiquitous mitochondrial oxidoreductase involved in apoptosis. FEBS Lett 476:118–123
11. Ekert PG, Vaux DL (2005) The mitochondrial death squad: hardened killers or innocent bystanders? Curr Opin Cell Biol 17:626–630
12. Schafer ZT, Kornbluth S (2006) The apoptosome: physiological, developmental, and pathological modes of regulation. Dev Cell 10:549–561
13. Lalier L, Cartron PF, Juin P et al (2007) Bax activation and mitochondrial insertion during apoptosis. Apoptosis 12:887–896
14. Ott M, Zhivotovsky B, Orrenius S (2007) Role of cardiolipin in cytochrome *c* release from mitochondria. Cell Death Differ 14:1243–1247

15. Iverson SL, Orrenius S (2004) The cardiolipin-cytochrome *c* interaction and the mitochondrial regulation of apoptosis. Arch Biochem Biophys 423:37–46
16. Scorrano L, Korsmeyer SJ (2003) Mechanisms of cytochrome *c* release by proapoptotic BCL-2 family members. Biochem Biophys Res Commun 304:437–444
17. Krajewski S, Krajewska M, Shabaik A, Miyashita T, Wang HG, Reed JC (1994) Immunohistochemical determination of in vivo distribution of Bax, a dominant inhibitor of Bcl-2. Am J Pathol 145:1323–1336
18. Kuwana T, Newmeyer DD (2003) Bcl-2-family proteins and the role of mitochondria in apoptosis. Curr Opin Cell Biol 15:691–699
19. Robertson JD, Enoksson M, Suomela M, Zhivotovsky B, Orrenius S (2002) Caspase-2 acts upstream of mitochondria to promote cytochrome *c* release during etoposide-induced apoptosis. J Biol Chem 277:29803–29809
20. Liston P, Fong WG, Korneluk RG (2003) The inhibitors of apoptosis: there is more to life than Bcl2. Oncogene 22:8568–8580
21. Ruchalski K, Mao H, Li Z et al (2006) Distinct hsp70 domains mediate apoptosis-inducing factor release and nuclear accumulation. J Biol Chem 281:7873–7880
22. Garrido C, Brunet M, Didelot C, Zermati Y, Schmitt E, Kroemer G (2006) Heat shock proteins 27 and 70: anti-apoptotic proteins with tumorigenic properties. Cell Cycle 5:2592–2601
23. Chandler D, el Naggar AK, Brisbay S, Redline RW, McDonnell TJ (1994) Apoptosis and expression of the bcl-2 proto-oncogene in the fetal and adult human kidney: evidence for the contribution of bcl-2 expression to renal carcinogenesis. Hum Pathol 25:789–796
24. Oberbauer R, Rohrmoser M, Regele H, Muhlbacher F, Mayer G (1999) Apoptosis of tubular epithelial cells in donor kidney biopsies predicts early renal allograft function. J Am Soc Nephrol 10:2006–2013
25. Kroning R, Katz D, Lichtenstein AK, Nagami GT (1999) Differential effects of cisplatin in proximal and distal renal tubule epithelial cell lines. Br J Cancer 79:293–299
26. Tarze A, Deniaud A, Le Bras M et al (2007) GAPDH, a novel regulator of the pro-apoptotic mitochondrial membrane permeabilization. Oncogene 26:2606–2620
27. Servais H, Jossin Y, Van Bambeke F, Tulkens PM, Mingeot-Leclercq MP (2006) Gentamicin causes apoptosis at low concentrations in renal LLC-PK1 cells subjected to electroporation. Antimicrob Agents Chemother 50:1213–1221
28. Justo P, Lorz C, Sanz A, Egido J, Ortiz A (2003) Intracellular mechanisms of cyclosporin A-induced tubular cell apoptosis. J Am Soc Nephrol 14:3072–3080
29. Wei Q, Dong G, Franklin J, Dong Z (2007) The pathological role of Bax in cisplatin nephrotoxicity. Kidney Int 72:53–62
30. Peherstorfer E, Mayer B, Boehm S et al (2002) Effects of microinjection of synthetic Bcl-2 domain peptides on apoptosis of renal tubular epithelial cells. Am J Physiol Renal Physiol 283:F190–F196
31. Sawada M, Hayes P, Matsuyama S (2003) Cytoprotective membrane-permeable peptides designed from the Bax-binding domain of Ku70. Nat Cell Biol 5:352–357
32. Retractions (2007). Nat Cell Biol 9:480
33. Justo P, Sanz AB, Egido J, Ortiz A (2005) 3,4-Dideoxyglucosone-3-ene induces apoptosis in renal tubular epithelial cells. Diabetes 54:2424–2429
34. Orlowski RZ (1999) The role of the ubiquitin-proteasome pathway in apoptosis. Cell Death Differ 6:303–313
35. Zhu H, Zhang L, Dong F et al (2005) Bik/NBK accumulation correlates with apoptosis-induction by bortezomib (PS-341, Velcade) and other proteasome inhibitors. Oncogene 24:4993–4999
36. Fribley A, Wang CY (2006) Proteasome inhibitor induces apoptosis through induction of endoplasmic reticulum stress. Cancer Biol Ther 5:745–748
37. Zavrski I, Kleeberg L, Kaiser M et al (2007) Proteasome as an emerging therapeutic target in cancer. Curr Pharm Des 13:471–485
38. Chwieralski CE, Welte T, Buhling F (2006) Cathepsin-regulated apoptosis. Apoptosis 11:143–149
39. Yuan XM, Li W, Dalen H et al (2002) Lysosomal destabilization in p53-induced apoptosis. Proc Natl Acad Sci USA 99:6286–6291
40. Cao X, Deng X, May WS (2003) Cleavage of Bax to p18 Bax accelerates stress-induced apoptosis, and a cathepsin-like protease may rapidly degrade p18 Bax. Blood 102:2605–2614
41. Szegezdi E, Logue SE, Gorman AM, Samali A (2006) Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep 7:880–885
42. Goetz JG, Nabi IR (2006) Interaction of the smooth endoplasmic reticulum and mitochondria. Biochem Soc Trans 34:370–373
43. Hetz C, Bernasconi P, Fisher J et al (2006) Proapoptotic BAX and BAK modulate the unfolded protein response by a direct interaction with IRE1alpha. Science 312:572–576
44. Mathai JP, Germain M, Shore GC (2005) BH3-only BIK regulates BAX,BAK-dependent release of Ca^{2+} from endoplasmic reticulum stores and mitochondrial apoptosis during stress-induced cell death. J Biol Chem 280:23829–23836
45. Zinszner H, Kuroda M, Wang X et al (1998) CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. Genes Dev 12:982–995
46. Erdal H, Berndtsson M, Castro J, Brunk U, Shoshan MC, Linder S (2005) Induction of lysosomal membrane permeabilization by compounds that activate p53-independent apoptosis. Proc Natl Acad Sci USA 102:192–197
47. Machamer CE (2003) Golgi disassembly in apoptosis: cause or effect? Trends Cell Biol 13:279–281
48. Hicks SW, Machamer CE (2005) Golgi structure in stress sensing and apoptosis. Biochim Biophys Acta 1744:406–414
49. Ortiz A, Lorz C, Catalan MP et al (2000) Expression of apoptosis regulatory proteins in tubular epithelium stressed in culture or following acute renal failure. Kidney Int 57:969–981
50. Kataoka T (2005) The caspase-8 modulator c-FLIP. Crit Rev Immunol 25:31–58
51. Lorz C, Justo P, Sanz AB, Egido J, Ortiz A (2005) Role of Bcl-xL in paracetamol-induced tubular epithelial cell death. Kidney Int 67:592–601
52. Modjtahedi N, Giordanetto F, Madeo F, Kroemer G (2006) Apoptosis-inducing factor: vital and lethal. Trends Cell Biol 16:264–272
53. Mao WP, Ye JL, Guan ZB et al (2007) Cadmium induces apoptosis in human embryonic kidney (HEK) 293 cells by caspase-dependent and -independent pathways acting on mitochondria. Toxicol In Vitro 21:343–354
54. Kagami S, Kondo S (2004) Beta1-integrins and glomerular injury. J Med Invest 51:1–13
55. Bijian K, Takano T, Papillon J, Khadir A, Cybulsky AV (2004) Extracellular matrix regulates glomerular epithelial cell survival and proliferation. Am J Physiol Renal Physiol 286:F255–F266
56. Dutta J, Fan Y, Gupta N, Fan G, Gelinas C (2006) Current insights into the regulation of programmed cell death by NF-kappaB. Oncogene 25:6800–6816
57. Park SJ, Jeong KS (2004) Cell-type-specific activation of mitogen-activated protein kinases in PAN-induced progressive renal disease in rats. Biochem Biophys Res Commun 323:1–8

58. Awazu M, Omori S, Hida M (2002) MAP kinase in renal development. Nephrol Dial Transplant 17(Suppl 9):5–7
59. Sen P, Mukherjee S, Ray D, Raha S (2003) Involvement of the Akt/PKB signaling pathway with disease processes. Mol Cell Biochem 253:241–246
60. O'Dea EL, Barken D, Peralta RQ et al (2007) A homeostatic model of IkappaB metabolism to control constitutive NF-kappaB activity. Mol Syst Biol 3:111
61. Heikaus S, Casliskan E, Mahotka C, Gabbert HE, Ramp U (2007) Differential gene expression in anticancer drug- and TRAIL-mediated apoptosis in renal cell carcinomas. Apoptosis 12:1645–1657
62. Shetty S, Gladden JB, Henson ES et al (2002) Tumor necrosis factor-related apoptosis inducing ligand (TRAIL) up-regulates death receptor 5 (DR5) mediated by NFkappaB activation in epithelial derived cell lines. Apoptosis 7:413–420
63. Efeyan A, Serrano M (2007) p53: guardian of the genome and policeman of the oncogenes. Cell Cycle 6:1006–1010
64. Vousden KH, Lane DP (2007) p53 in health and disease. Nat Rev Mol Cell Biol 8:275–283
65. Ries F, Klastersky J (1986) Nephrotoxicity induced by cancer chemotherapy with special emphasis on cisplatin toxicity. Am J Kidney Dis 8:368–379
66. Kuhlmann MK, Burkhardt G, Kohler H (1997) Insights into potential cellular mechanisms of cisplatin nephrotoxicity and their clinical application. Nephrol Dial Transplant 12:2478–2480
67. Endo T, Kimura O, Sakata M (2000) Carrier-mediated uptake of cisplatin by the OK renal epithelial cell line. Toxicology 146:187–195
68. Ban M, Hettich D, Huguet N (1994) Nephrotoxicity mechanism of *cis*-platinum(II) diamine dichloride in mice. Toxicol Lett 71:161–168
69. Huang Q, Dunn RT, Jayadev S et al (2001) Assessment of cisplatin-induced nephrotoxicity by microarray technology. Toxicol Sci 63:196–207
70. Arany I, Safirstein RL (2003) Cisplatin nephrotoxicity. Semin Nephrol 23:460–464
71. Arany I, Megyesi JK, Kaneto H, Price PM, Safirstein RL (2004) Cisplatin-induced cell death is EGFR/src/ERK signaling dependent in mouse proximal tubule cells. Am J Physiol Renal Physiol 287:F543–F549
72. Dobyan DC, Levi J, Jacobs C, Kosek J, Weiner MW (1980) Mechanism of *cis*-platinum nephrotoxicity. II. Morphologic observations. J Pharmacol Exp Ther 213:551–556
73. Kaushal GP, Kaushal V, Hong X, Shah SV (2001) Role and regulation of activation of caspases in cisplatin-induced injury to renal tubular epithelial cells. Kidney Int 60:1726–1736
74. Wei Q, Dong G, Yang T, Megyesi J, Price PM, Dong Z (2007) Activation and Involvement of p53 in Cisplatin-induced Nephrotoxicity. Am J Physiol Renal Physiol 293:F1282–F1291
75. Jiang M, Pabla N, Murphy RF et al (2007) Nutlin-3 protects kidney cells during cisplatin therapy by suppressing Bax/Bak activation. J Biol Chem 282:2636–2645
76. Wang J, Pabla N, Wang CY, Wang W, Schoenlein PV, Dong Z (2006) Caspase-mediated cleavage of ATM during cisplatin-induced tubular cell apoptosis: inactivation of its kinase activity toward p53. Am J Physiol Renal Physiol 291:F1300–F1307
77. Dippold WG, Jay G, DeLeo AB, Khoury G, Old LJ (1981) p53 transformation-related protein: detection by monoclonal antibody in mouse and human cells. Proc Natl Acad Sci USA 78:1695–1699
78. Jiang M, Wei Q, Wang J et al (2006) Regulation of PUMA-alpha by p53 in cisplatin-induced renal cell apoptosis. Oncogene 25:4056–4066
79. Seth R, Yang C, Kaushal V, Shah SV, Kaushal GP (2005) p53-dependent caspase-2 activation in mitochondrial release of apoptosis-inducing factor and its role in renal tubular epithelial cell injury. J Biol Chem 280:31230–31239
80. Moll UM, Wolff S, Speidel D, Deppert W (2005) Transcription-independent pro-apoptotic functions of p53. Curr Opin Cell Biol 17:631–636
81. Ramesh G, Reeves WB (2005) p38 MAP kinase inhibition ameliorates cisplatin nephrotoxicity in mice. Am J Physiol Renal Physiol 289:F166–F174
82. Kim YK, Jung JS, Lee SH, Kim YW (1997) Effects of antioxidants and Ca^{2+} in cisplatin-induced cell injury in rabbit renal cortical slices. Toxicol Appl Pharmacol 146:261–269
83. Zhou H, Kato A, Yasuda H et al (2004) The induction of cell cycle regulatory and DNA repair proteins in cisplatin-induced acute renal failure. Toxicol Appl Pharmacol 200:111–120
84. Price PM, Yu F, Kaldis P et al (2006) Dependence of cisplatin-induced cell death in vitro and in vivo on cyclin-dependent kinase 2. J Am Soc Nephrol 17:2434–2442
85. Yu F, Megyesi J, Safirstein RL, Price PM (2007) Involvement of the CDK2-E2F1 pathway in cisplatin cytotoxicity in vitro and in vivo. Am J Physiol Renal Physiol 293:F52–F59
86. Momekov G, Konstantinov S, Topashka-Ancheva M, Bakalova A, Arpadjan S, Karaivanova M (2007) Cellular pharmacology, antineoplastic activity and low in vivo toxicity of a carboxylato-bridged platinum(II) complex bis(acetato)diammine-bis-micro-acetato diplatinum(II) dihydrate. Med Chem 3:157–165
87. Mingeot-Leclercq MP, Tulkens PM (1999) Aminoglycosides: nephrotoxicity. Antimicrob Agents Chemother 43:1003–1012
88. Fabre J, Rudhardt M, Blanchard P, Regamey C (1976) Persistence of sisomicin and gentamicin in renal cortex and medulla compared with other organs and serum of rats. Kidney Int 10:444–449
89. Vandewalle A, Farman N, Morin JP, Fillastre JP, Hatt PY, Bonvalet JP (1981) Gentamicin incorporation along the nephron: autoradiographic study on isolated tubules. Kidney Int 19:529–539
90. Sastrasinh M, Knauss TC, Weinberg JM, Humes HD (1982) Identification of the aminoglycoside binding site in rat renal brush border membranes. J Pharmacol Exp Ther 222:350–358
91. Moestrup SK, Cui S, Vorum H et al (1995) Evidence that epithelial glycoprotein 330/megalin mediates uptake of polybasic drugs. J Clin Invest 96:1404–1413
92. Silverblatt FJ, Kuehn C (1979) Autoradiography of gentamicin uptake by the rat proximal tubule cell. Kidney Int 15:335–345
93. Giurgea-Marion L, Toubeau G, Laurent G, Heuson-Stiennon JA, Tulkens PM (1986) Impairment of lysosome-pinocytic vesicle fusion in rat kidney proximal tubules after treatment with gentamicin at low doses. Toxicol Appl Pharmacol 86:271–285
94. El Mouedden M, Laurent G, Mingeot-Leclercq MP, Taper HS, Cumps J, Tulkens PM (2000) Apoptosis in renal proximal tubules of rats treated with low doses of aminoglycosides. Antimicrob Agents Chemother 44:665–675
95. El Mouedden M, Laurent G, Mingeot-Leclercq MP, Tulkens PM (2000) Gentamicin-induced apoptosis in renal cell lines and embryonic rat fibroblasts. Toxicol Sci 56:229–239
96. Sandoval RM, Molitoris BA (2004) Gentamicin traffics retrograde through the secretory pathway and is released in the cytosol via the endoplasmic reticulum. Am J Physiol Renal Physiol 286:F617–F624
97. Mather M, Rottenberg H (2001) Polycations induce the release of soluble intermembrane mitochondrial proteins. Biochim Biophys Acta 1503:357–368
98. Horibe T, Matsui H, Tanaka M et al (2004) Gentamicin binds to the lectin site of calreticulin and inhibits its chaperone activity. Biochem Biophys Res Commun 323:281–287

99. Lesniak W, Pecoraro VL, Schacht J (2005) Ternary complexes of gentamicin with iron and lipid catalyze formation of reactive oxygen species. Chem Res Toxicol 18:357–364
100. Li C, Lim SW, Sun BK, Yang CW (2004) Chronic cyclosporine nephrotoxicity: new insights and preventive strategies. Yonsei Med J 45:1004–1016
101. Fellstrom B (2004) Cyclosporine nephrotoxicity. Transplant Proc 36:220S–223S
102. Stratta P, Canavese C, Quaglia M et al (2005) Posttransplantation chronic renal damage in nonrenal transplant recipients. Kidney Int 68:1453–1463
103. Olyaei AJ, de Mattos AM, Bennett WM (2001) Nephrotoxicity of immunosuppressive drugs: new insight and preventive strategies. Curr Opin Crit Care 7:384–389
104. Dieperink H, Kemp E, Leyssac PP et al (1986) Ketoconazole and cyclosporine A: combined effects on rat renal function and on serum and tissue cyclosporine A concentration. Clin Nephrol 25(Suppl 1):S137–S143
105. del Moral RG, Olmo A, Aguilar M, O'Valle F (1998) P glycoprotein: a new mechanism to control drug-induced nephrotoxicity. Exp Nephrol 6:89–97
106. Hauser IA, Schaeffeler E, Gauer S et al (2005) ABCB1 genotype of the donor but not of the recipient is a major risk factor for cyclosporine-related nephrotoxicity after renal transplantation. J Am Soc Nephrol 16:1501–1511
107. Thomas SE, Andoh TF, Pichler RH et al (1998) Accelerated apoptosis characterizes cyclosporine-associated interstitial fibrosis. Kidney Int 53:897–908
108. Shimizu H, Takahashi M, Takeda S et al (2004) Conversion from cyclosporine A to mycophenolate mofetil protects recipient kidney and prevents intimal hyperplasia in rat aortic allografts. Transpl Immunol 13:219–227
109. Lee SY, Jo SK, Cho WY, Kim HK, Won NH (2004) The effect of alpha-melanocyte-stimulating hormone on renal tubular cell apoptosis and tubulointerstitial fibrosis in cyclosporine A nephrotoxicity. Transplantation 78:1756–1764
110. Ortiz A, Lorz C, Catalan M, Ortiz A, Coca S, Egido J (1998) Cyclosporine A induces apoptosis in murine tubular epithelial cells: role of caspases. Kidney Int Suppl 68:S25–S29
111. Amore A, Emancipator SN, Cirina P et al (2000) Nitric oxide mediates cyclosporine-induced apoptosis in cultured renal cells. Kidney Int 57:1549–1559
112. Jeon SH, Piao YJ, Choi KJ et al (2005) Prednisolone suppresses cyclosporin A-induced apoptosis but not cell cycle arrest in MDCK cells. Arch Biochem Biophys 435:382–392
113. Han SY, Chang EJ, Choi HJ et al (2006) Apoptosis by cyclosporine in mesangial cells. Transplant Proc 38:2244–2246
114. Yang CW, Faulkner GR, Wahba IM et al (2002) Expression of apoptosis-related genes in chronic cyclosporine nephrotoxicity in mice. Am J Transplant 2:391–399
115. Shihab FS, Bennett WM, Yi H, Andoh TF (2005) Effect of pirfenidone on apoptosis-regulatory genes in chronic cyclosporine nephrotoxicity. Transplantation 79:419–426
116. Kim SI, Song HY, Hwang JH et al (2000) Cyclosporine nephrotoxicity: the mechanisms of cell injury by cyclosporine A in renal proximal tubular cells. Transplant Proc 32:1621–1622
117. Fischer U, Schulze-Osthoff K (2005) New approaches and therapeutics targeting apoptosis in disease. Pharmacol Rev 57:187–215
118. Lorz C, Benito-Martin A, Justo P et al (2006) Modulation of renal tubular cell survival: where is the evidence? Curr Med Chem 13:449–454
119. Ciarimboli G, Ludwig T, Lang D et al (2005) Cisplatin nephrotoxicity is critically mediated via the human organic cation transporter 2. Am J Pathol 167:1477–1484
120. Cihlar T, Ho ES, Lin DC, Mulato AS (2001) Human renal organic anion transporter 1 (hOAT1) and its role in the nephrotoxicity of antiviral nucleotide analogs. Nucleosides Nucleotides Nucleic Acids 20:641–648
121. Nagai J, Takano M (2004) Molecular aspects of renal handling of aminoglycosides and strategies for preventing the nephrotoxicity. Drug Metab Pharmacokinet 19:159–170
122. Takamoto K, Kawada M, Ikeda D, Yoshida M (2005) Apolipoprotein E3 (apoE3) safeguards pig proximal tubular LLC-PK1 cells against reduction in SGLT1 activity induced by gentamicin C. Biochim Biophys Acta 1722:247–253
123. Perez M, Castilla M, Torres AM, Lazaro JA, Sarmiento E, Tejedor A (2004) Inhibition of brush border dipeptidase with cilastatin reduces toxic accumulation of cyclosporin A in kidney proximal tubule epithelial cells. Nephrol Dial Transplant 19:2445–2455
124. Pingle SC, Mishra S, Marcuzzi A et al (2004) Osmotic diuretics induce adenosine A1 receptor expression and protect renal proximal tubular epithelial cells against cisplatin-mediated apoptosis. J Biol Chem 279:43157–43167
125. Letai A (2005) Pharmacological manipulation of Bcl-2 family members to control cell death. J Clin Invest 115:2648–2655
126. Gama V, Yoshida T, Gomez JA et al (2006) Involvement of the ubiquitin pathway in decreasing Ku70 levels in response to drug-induced apoptosis. Exp Cell Res 312:488–499
127. Wang J, Wei Q, Wang CY, Hill WD, Hess DC, Dong Z (2004) Minocycline up-regulates Bcl-2 and protects against cell death in mitochondria. J Biol Chem 279:19948–19954
128. Wada T, Pippin JW, Marshall CB, Griffin SV, Shankland SJ (2005) Dexamethasone prevents podocyte apoptosis induced by puromycin aminonucleoside: role of p53 and Bcl-2-related family proteins. J Am Soc Nephrol 16:2615–2625
129. Wilson C, Foster GH, Bitzan M (2005) Silencing of Bak ameliorates apoptosis of human proximal tubular epithelial cells by Escherichia coli-derived Shiga toxin 2. Infection 33:362–367
130. Zheng X, Zhang X, Sun H et al (2006) Protection of renal ischemia injury using combination gene silencing of complement 3 and caspase 3 genes. Transplantation 82:1781–1786
131. Du C, Wang S, Diao H, Guan Q, Zhong R, Jevnikar AM (2006) Increasing resistance of tubular epithelial cells to apoptosis by shRNA therapy ameliorates renal ischemia-reperfusion injury. Am J Transplant 6:2256–2267
132. Basnakian AG, Kaushal GP, Shah SV (2002) Apoptotic pathways of oxidative damage to renal tubular epithelial cells. Antioxid Redox Signal 4:915–924
133. Shirwaikar A, Malini S, Kumari SC (2003) Protective effect of Pongamia pinnata flowers against cisplatin and gentamicin induced nephrotoxicity in rats. Indian J Exp Biol 41:58–62
134. Juan SH, Chen CH, Hsu YH et al (2007) Tetramethylpyrazine protects rat renal tubular cell apoptosis induced by gentamicin. Nephrol Dial Transplant 22:732–739
135. Ray SD, Patel D, Wong V, Bagchi D (2000) In vivo protection of dna damage associated apoptotic and necrotic cell deaths during acetaminophen-induced nephrotoxicity, amiodarone-induced lung toxicity and doxorubicin-induced cardiotoxicity by a novel IH636 grape seed proanthocyanidin extract. Res Commun Mol Pathol Pharmacol 107:137–166
136. Al Ghamdi SS, Chatterjee PK, Raftery MJ, Thiemermann C, Yaqoob MM (2004) Role of cytochrome P4502E1 activation in proximal tubular cell injury induced by hydrogen peroxide. Ren Fail 26:103–110
137. Liu H, Baliga R (2003) Cytochrome P450 2E1 null mice provide novel protection against cisplatin-induced nephrotoxicity and apoptosis. Kidney Int 63:1687–1696
138. Messaris E, Antonakis PT, Memos N, Chatzigianni E, Leandros E, Konstadoulakis MM (2004) Deferoxamine administration in

septic animals: improved survival and altered apoptotic gene expression. Int Immunopharmacol 4:455–459
139. Ramesh G, Reeves WB (2003) TNFR2-mediated apoptosis and necrosis in cisplatin-induced acute renal failure. Am J Physiol Renal Physiol 285:F610–F618
140. Kim YK, Choi TR, Kwon CH, Kim JH, Woo JS, Jung JS (2003) Beneficial effect of pentoxifylline on cisplatin-induced acute renal failure in rabbits. Ren Fail 25:909–922
141. Falk R, Hacham M, Nyska A, Foley JF, Domb AJ, Polacheck I (2005) Induction of interleukin-1beta, tumour necrosis factor-alpha and apoptosis in mouse organs by amphotericin B is neutralized by conjugation with arabinogalactan. J Antimicrob Chemother 55:713–720
142. Shiozaki EN, Shi Y (2004) Caspases, IAPs and Smac/DIABLO: mechanisms from structural biology. Trends Biochem Sci 29:486–494
143. Yang B, El Nahas AM, Fisher M et al (2004) Inhibitors directed towards caspase-1 and –3 are less effective than pan caspase inhibition in preventing renal proximal tubular cell apoptosis. Nephron Exp Nephrol 96:e39–e51
144. Ortiz A, Justo P, Sanz A et al (2005) Tubular cell apoptosis and cidofovir-induced acute renal failure. Antivir Ther 10:185–190
145. Yang W, Guastella J, Huang JC et al (2003) MX1013, a dipeptide caspase inhibitor with potent in vivo antiapoptotic activity. Br J Pharmacol 140:402–412
146. Cai SX, Guan L, Jia S et al (2004) Dipeptidyl aspartyl fluoromethylketones as potent caspase inhibitors: SAR of the N-protecting group. Bioorg Med Chem Lett 14:5295–5300
147. Tao Y, Kim J, Faubel S et al (2005) Caspase inhibition reduces tubular apoptosis and proliferation and slows disease progression in polycystic kidney disease. Proc Natl Acad Sci USA 102:6954–6959
148. Fischer U, Janicke RU, Schulze-Osthoff K (2003) Many cuts to ruin: a comprehensive update of caspase substrates. Cell Death Differ 10:76–100
149. Cilenti L, Kyriazis GA, Soundarapandian MM et al (2005) Omi/HtrA2 protease mediates cisplatin-induced cell death in renal cells. Am J Physiol Renal Physiol 288:F371–F379
150. Jo SK, Cho WY, Sung SA, Kim HK, Won NH (2005) MEK inhibitor, U0126, attenuates cisplatin-induced renal injury by decreasing inflammation and apoptosis. Kidney Int 67:458–466
151. Imai E, Isaka Y (2002) Targeting growth factors to the kidney: myth or reality? Curr Opin Nephrol Hypertens 11:49–57
152. Ramesh G, Reeves WB (2004) Inflammatory cytokines in acute renal failure. Kidney Int Suppl (91):S56–S61
153. Patrick DM, Leone AK, Shellenberger JJ, Dudowicz KA, King JM (2006) Proinflammatory cytokines tumor necrosis factor-alpha and interferon-gamma modulate epithelial barrier function in Madin-Darby canine kidney cells through mitogen activated protein kinase signaling. BMC Physiol 6:2
154. Jung JY, Song JH, Li C et al (2005) Expression of epidermal growth factor in the developing rat kidney. Am J Physiol Renal Physiol 288:F227–F235
155. Coimbra TM, Cieslinski DA, Humes HD (1990) Epidermal growth factor accelerates renal repair in mercuric chloride nephrotoxicity. Am J Physiol 259:F438–F443
156. Daemen MA, van't Veer C, Denecker G et al (1999) Inhibition of apoptosis induced by ischemia-reperfusion prevents inflammation. J Clin Invest 104:541–549
157. Varlam DE, Siddiq MM, Parton LA, Russmann H (2001) Apoptosis contributes to amphotericin B-induced nephrotoxicity. Antimicrob Agents Chemother 45:679–685
158. Ernst F, Hetzel S, Stracke S et al (2001) Renal proximal tubular cell growth and differentiation are differentially modulated by renotropic growth factors and tyrosine kinase inhibitors. Eur J Clin Invest 31:1029–1039
159. Mizui M, Isaka Y, Takabatake Y et al (2004) Electroporation-mediated HGF gene transfer ameliorated cyclosporine nephrotoxicity. Kidney Int 65:2041–2053
160. Kiley SC, Thornhill BA, Tang SS, Ingelfinger JR, Chevalier RL (2003) Growth factor-mediated phosphorylation of proapoptotic BAD reduces tubule cell death in vitro and in vivo. Kidney Int 63:33–42
161. Nishida M, Fujimoto S, Toiyama K, Sato H, Hamaoka K (2004) Effect of hematopoietic cytokines on renal function in cisplatin-induced ARF in mice. Biochem Biophys Res Commun 324:341–347
162. Alvarez Arroyo MV, Suzuki Y, Yague S et al (2002) Role of endogenous vascular endothelial growth factor in tubular cell protection against acute cyclosporine toxicity. Transplantation 74:1618–1624
163. Hiromura K, Pippin JW, Fero ML, Roberts JM, Shankland SJ (1999) Modulation of apoptosis by the cyclin-dependent kinase inhibitor p27(Kip1). J Clin Invest 103:597–604
164. Bukanov NO, Smith LA, Klinger KW, Ledbetter SR, Ibraghimov-Beskrovnaya O (2006) Long-lasting arrest of murine polycystic kidney disease with CDK inhibitor roscovitine. Nature 444:949–952
165. Price PM, Safirstein RL, Megyesi J (2004) Protection of renal cells from cisplatin toxicity by cell cycle inhibitors. Am J Physiol Renal Physiol 286:F378–F384
166. Charlwood J, Skehel JM, King N et al (2002) Proteomic analysis of rat kidney cortex following treatment with gentamicin. J Proteome Res 1:73–82
167. Amin RP, Vickers AE, Sistare F et al (2004) Identification of putative gene based markers of renal toxicity. Environ Health Perspect 112:465–479
168. Kramer JA, Pettit SD, Amin RP et al (2004) Overview on the application of transcription profiling using selected nephrotoxicants for toxicology assessment. Environ Health Perspect 112:460–464
169. Aicher L, Wahl D, Arce A, Grenet O, Steiner S (1998) New insights into cyclosporine A nephrotoxicity by proteome analysis. Electrophoresis 19:1998–2003
170. Bandara LR, Kelly MD, Lock EA, Kennedy S (2003) A correlation between a proteomic evaluation and conventional measurements in the assessment of renal proximal tubular toxicity. Toxicol Sci 73:195–206
171. Vickers AE, Rose K, Fisher R, Saulnier M, Sahota P, Bentley P (2004) Kidney slices of human and rat to characterize cisplatin-induced injury on cellular pathways and morphology. Toxicol Pathol 32:577–590
172. Thompson KL, Afshari CA, Amin RP et al (2004) Identification of platform-independent gene expression markers of cisplatin nephrotoxicity. Environ Health Perspect 112:488–494
173. Bleasby K, Hall LA, Perry JL, Mohrenweiser HW, Pritchard JB (2005) Functional consequences of single nucleotide polymorphisms in the human organic anion transporter hOAT1 (SLC22A6). J Pharmacol Exp Ther 314:923–931
174. Lorz C, Justo P, Sanz A, Subira D, Egido J, Ortiz A (2004) Paracetamol-induced renal tubular injury: a role for ER stress. J Am Soc Nephrol 15:380–389
175. Zhang J, Clark JR Jr, Herman EH, Ferrans VJ (1996) Doxorubicin-induced apoptosis in spontaneously hypertensive rats: differential effects in heart, kidney and intestine, and inhibition by ICRF-187. J Mol Cell Cardiol 28:1931–1943
176. Cheng CY, Sue YM, Chen CH et al (2006) Tetramethylpyrazine attenuates adriamycin-induced apoptotic injury in rat renal tubular cells NRK-52E. Planta Med 72:888–893
177. Aravindan N, Cata JP, Hoffman L et al (2006) Effects of isoflurane, pentobarbital, and urethane on apoptosis and apoptotic

signal transduction in rat kidney. Acta Anaesthesiol Scand 50:1229–1237
178. Thevenod F (2003) Nephrotoxicity and the proximal tubule. Insights from cadmium. Nephron Physiol 93:87–93
179. Mejia-Villet JM, Ramirez V, Cruz C, Uribe N, Gamba G, Bobadilla NA (2007) Renal ischemia/reperfusion injury is prevented by the mineralocorticoid receptor blocker spironolactone. Am J Physiol Renal Physiol 293:F78–F86
180. Takeda M, Kobayashi M, Shirato I, Osaki T, Endou H (1997) Cisplatin-induced apoptosis of immortalized mouse proximal tubule cells is mediated by interleukin-1 beta converting enzyme (ICE) family of proteases but inhibited by overexpression of Bcl-2. Arch Toxicol 71:612–621
181. Takeda M, Kobayashi M, Shirato I, Endou H (1998) Involvement of macromolecule synthesis, endonuclease activation and c-fos expression in cisplatin-induced apoptosis of mouse proximal tubule cells. Toxicol Lett 94:83–92
182. Sheikh-Hamad D, Nadkarni V, Choi YJ et al (2001) Cyclosporine A inhibits the adaptive responses to hypertonicity: a potential mechanism of nephrotoxicity. J Am Soc Nephrol 12:2732–2741
183. Lantum HB, Baggs RB, Krenitsky DM, Anders MW (2002) Nephrotoxicity of chlorofluoroacetic acid in rats. Toxicol Sci 70:261–268
184. Hickey EJ, Raje RR, Reid VE, Gross SM, Ray SD (2001) Diclofenac induced in vivo nephrotoxicity may involve oxidative stress-mediated massive genomic DNA fragmentation and apoptotic cell death. Free Radic Biol Med 31:139–152
185. Creydt VP, Silberstein C, Zotta E, Ibarra C (2006) Cytotoxic effect of Shiga toxin-2 holotoxin and its B subunit on human renal tubular epithelial cells. Microbes Infect 8:410–419
186. Karpman D, Hakansson A, Perez MT et al (1998) Apoptosis of renal cortical cells in the hemolytic-uremic syndrome: in vivo and in vitro studies. Infect Immun 66:636–644
187. Cunningham PN, Dyanov HM, Park P, Wang J, Newell KA, Quigg RJ (2002) Acute renal failure in endotoxemia is caused by TNF acting directly on TNF receptor-1 in kidney. J Immunol 168:5817–5823
188. Cunningham PN, Wang Y, Guo R, He G, Quigg RJ (2004) Role of Toll-like receptor 4 in endotoxin-induced acute renal failure. J Immunol 172:2629–2635
189. Dharnidharka VR, Nadeau K, Cannon CL, Harris HW, Rosen S (1998) Ciprofloxacin overdose: acute renal failure with prominent apoptotic changes. Am J Kidney Dis 31:710–712
190. Duncan-Achanzar KB, Jones JT, Burke MF, Carter DE, Laird HE (1996) Inorganic mercury chloride-induced apoptosis in the cultured porcine renal cell line LLC-PK1. J Pharmacol Exp Ther 277:1726–1732
191. Milutinovic A, Zivin M, Zorc-Pleskovic R, Sedmak B, Suput D (2003) Nephrotoxic effects of chronic administration of microcystins -LR and -YR. Toxicon 42:281–288
192. Kamp HG, Eisenbrand G, Schlatter J, Wurth K, Janzowski C (2005) Ochratoxin A: induction of (oxidative) DNA damage, cytotoxicity and apoptosis in mammalian cell lines and primary cells. Toxicology 206:413–425
193. Sauvant C, Holzinger H, Gekle M (2005) Proximal tubular toxicity of ochratoxin A is amplified by simultaneous inhibition of the extracellular signal-regulated kinases 1/2. J Pharmacol Exp Ther 313:234–241
194. Cao LC, Honeyman TW, Cooney R, Kennington L, Scheid CR, Jonassen JA (2004) Mitochondrial dysfunction is a primary event in renal cell oxalate toxicity. Kidney Int 66:1890–1900
195. Duan S, Zhou X, Liu F et al (2006) Comparative cytotoxicity of high-osmolar and low-osmolar contrast media on HKCs in vitro. J Nephrol 19:717–724
196. Hizoh I, Strater J, Schick CS, Kubler W, Haller C (1998) Radiocontrast-induced DNA fragmentation of renal tubular cells in vitro: role of hypertonicity. Nephrol Dial Transplant 13:911–918
197. Garofalo AS, Borges FT, Dalboni MA, Pavao dos Santos OF (2007) Reactive oxygen species independent cytotoxicity induced by radiocontrast agents in tubular cells (LLC-PK1 and MDCK). Ren Fail 29:121–131
198. Itoh Y, Yano T, Sendo T et al (2006) Involvement of de novo ceramide synthesis in radiocontrast-induced renal tubular cell injury. Kidney Int 69:288–297
199. Lieberthal W, Fuhro R, Andry CC et al (2001) Rapamycin impairs recovery from acute renal failure: role of cell-cycle arrest and apoptosis of tubular cells. Am J Physiol Renal Physiol 281:F693–F706
200. Blanco-Colio LM, Justo P, Daehn I, Lorz C, Ortiz A, Egido J (2003) Bcl-xL overexpression protects from apoptosis induced by HMG-CoA reductase inhibitors in murine tubular cells. Kidney Int 64:181–191
201. Loffing J, Loffing-Cueni D, Hegyi I et al (1996) Thiazide treatment of rats provokes apoptosis in distal tubule cells. Kidney Int 50:1180–1190
202. Markowitz GS, Fine PL, Stack JI et al (2003) Toxic acute tubular necrosis following treatment with zoledronate (Zometa). Kidney Int 64:281–289
203. Chen CH, Lin H, Hsu YH et al (2006) The protective effect of prostacyclin on adriamycin-induced apoptosis in rat renal tubular cells. Eur J Pharmacol 529:8–15
204. Schwerdt G, Freudinger R, Schuster C, Weber F, Thews O, Gekle M (2005) Cisplatin-induced apoptosis is enhanced by hypoxia and by inhibition of mitochondria in renal collecting duct cells. Toxicol Sci 85:735–742
205. Sheikh-Hamad D, Cacini W, Buckley AR et al (2004) Cellular and molecular studies on cisplatin-induced apoptotic cell death in rat kidney. Arch Toxicol 78:147–155
206. Baek SM, Kwon CH, Kim JH, Woo JS, Jung JS, Kim YK (2003) Differential roles of hydrogen peroxide and hydroxyl radical in cisplatin-induced cell death in renal proximal tubular epithelial cells. J Lab Clin Med 142:178–186
207. Satoh M, Kashihara N, Fujimoto S et al (2003) A novel free radical scavenger, edarabone, protects against cisplatin-induced acute renal damage in vitro and in vivo. J Pharmacol Exp Ther 305:1183–1190
208. Park MS, De Leon M, Devarajan P (2002) Cisplatin induces apoptosis in LLC-PK1 cells via activation of mitochondrial pathways. J Am Soc Nephrol 13:858–865
209. Davis CA, Nick HS, Agarwal A (2001) Manganese superoxide dismutase attenuates Cisplatin-induced renal injury: importance of superoxide. J Am Soc Nephrol 12:2683–2690
210. Kruidering M, van de WB, de Heer E, Mulder GJ, Nagelkerke JF (1997) Cisplatin-induced nephrotoxicity in porcine proximal tubular cells: mitochondrial dysfunction by inhibition of complexes I to IV of the respiratory chain. J Pharmacol Exp Ther 280:638–649
211. Zhang J, Duarte CG, Ellis S (1999) Contrast medium- and mannitol-induced apoptosis in heart and kidney of SHR rats. Toxicol Pathol 27:427–435
212. Jin QH, Zhao B, Zhang XJ (2004) Cytochrome *c* release and endoplasmic reticulum stress are involved in caspase-dependent apoptosis induced by G418. Cell Mol Life Sci 61:1816–1825
213. Cummings BS, McHowat J, Schnellmann RG (2004) Role of an endoplasmic reticulum Ca^{2+}-independent phospholipase A2 in cisplatin-induced renal cell apoptosis. J Pharmacol Exp Ther 308:921–928
214. Tervahartiala P, Kivisaari L, Kivisaari R, Vehmas T, Virtanen I (1997) Structural changes in the renal proximal tubular cells induced by iodinated contrast media. Nephron 76:96–102

215. Humes HD, Sastrasinh M, Weinberg JM (1984) Calcium is a competitive inhibitor of gentamicin-renal membrane binding interactions and dietary calcium supplementation protects against gentamicin nephrotoxicity. J Clin Invest 73:134–147
216. Giuliano RA, Verpooten GA, Verbist L, Wedeen RP, De Broe ME (1986) In vivo uptake kinetics of aminoglycosides in the kidney cortex of rats. J Pharmacol Exp Ther 236:470–475
217. Oyama Y, Takeda T, Hama H et al (2005) Evidence for megalin-mediated proximal tubular uptake of L-FABP, a carrier of potentially nephrotoxic molecules. Lab Invest 85:522–531
218. Chiu PJ, Miller GH, Long JF, Waitz JA (1979) Renal uptake and nephrotoxicity of gentamicin during urinary alkalinization in rats. Clin Exp Pharmacol Physiol 6:317–326
219. Xiao T, Choudhary S, Zhang W, Ansari NH, Salahudeen A (2003) Possible involvement of oxidative stress in cisplatin-induced apoptosis in LLC-PK1 cells. J Toxicol Environ Health A 66:469–479
220. Tayem Y, Johnson TR, Mann BE, Green CJ, Motterlini R (2006) Protection against cisplatin-induced nephrotoxicity by a carbon monoxide-releasing molecule. Am J Physiol Renal Physiol 290:F789–F794
221. Nagothu KK, Bhatt R, Kaushal GP, Portilla D (2005) Fibrate prevents cisplatin-induced proximal tubule cell death. Kidney Int 68:2680–2693
222. Lee S, Kim W, Moon SO et al (2006) Rosiglitazone ameliorates cisplatin-induced renal injury in mice. Nephrol Dial Transplant 21:2096–2105
223. Francescato HD, Costa RS, Scavone C, Coimbra TM (2007) Parthenolide reduces cisplatin-induced renal damage. Toxicology 230:64–75
224. Okuda M, Masaki K, Fukatsu S, Hashimoto Y, Inui K (2000) Role of apoptosis in cisplatin-induced toxicity in the renal epithelial cell line LLC-PK1. Implication of the functions of apical membranes. Biochem Pharmacol 59:195–201
225. Iguchi T, Nishikawa M, Chang B et al (2004) Edaravone inhibits acute renal injury and cyst formation in cisplatin-treated rat kidney. Free Radic Res 38:333–341
226. Sueishi K, Mishima K, Makino K et al (2002) Protection by a radical scavenger edaravone against cisplatin-induced nephrotoxicity in rats. Eur J Pharmacol 451:203–208
227. Santos NA, Bezerra CS, Martins NM, Curti C, Bianchi ML, Santos AC (2007) Hydroxyl radical scavenger ameliorates cisplatin-induced nephrotoxicity by preventing oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. Cancer Chemother Pharmacol 61:145–155
228. Lee S, Moon SO, Kim W et al (2006) Protective role of L-2-oxothiazolidine-4-carboxylic acid in cisplatin-induced renal injury. Nephrol Dial Transplant 21:2085–2095
229. Ikari A, Nagatani Y, Tsukimoto M, Harada H, Miwa M, Takagi K (2005) Sodium-dependent glucose transporter reduces peroxynitrite and cell injury caused by cisplatin in renal tubular epithelial cells. Biochim Biophys Acta 1717:109–117
230. Martinez-Salgado C, Eleno N, Morales AI, Perez-Barriocanal F, Arevalo M, Lopez-Novoa JM (2004) Gentamicin treatment induces simultaneous mesangial proliferation and apoptosis in rats. Kidney Int 65:2161–2171
231. Parlakpinar H, Tasdemir S, Polat A et al (2006) Protective effect of chelerythrine on gentamicin-induced nephrotoxicity. Cell Biochem Funct 24:41–48
232. Bledsoe G, Crickman S, Mao J et al (2006) Kallikrein/kinin protects against gentamicin-induced nephrotoxicity by inhibition of inflammation and apoptosis. Nephrol Dial Transplant 21:624–633
233. Spargias K, Alexopoulos E, Kyrzopoulos S et al (2004) Ascorbic acid prevents contrast-mediated nephropathy in patients with renal dysfunction undergoing coronary angiography or intervention. Circulation 110:2837–2842
234. Itoh Y, Yano T, Sendo T, Oishi R (2005) Clinical and experimental evidence for prevention of acute renal failure induced by radiographic contrast media. J Pharmacol Sci 97:473–488
235. Gibson EM, Henson ES, Haney N, Villanueva J, Gibson SB (2002) Epidermal growth factor protects epithelial-derived cells from tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis by inhibiting cytochrome *c* release. Cancer Res 62:488–496
236. Liu Y, Sun AM, Dworkin LD (1998) Hepatocyte growth factor protects renal epithelial cells from apoptotic cell death. Biochem Biophys Res Commun 246:821–826
237. Ling H, Li X, Jha S et al (2003) Therapeutic role of TGF-beta-neutralizing antibody in mouse cyclosporin A nephropathy: morphologic improvement associated with functional preservation. J Am Soc Nephrol 14:377–388