

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

H. Servais · A. Ortiz · O. Devuyst · S. Denamur · P. M. Tulkens · M.-P. Mingeot-Leclercq

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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 exam-

ined. 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-1 β -converting enzyme
GADD	Growth arrest and DNA damage-inducible
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-1 α	Inositol-requiring enzyme 1alpha
JNKs	Janus kinases

H. Servais · S. Denamur · P. M. Tulkens · M.-P. Mingeot-Leclercq (✉)
Faculty of Medicine, Unité de Pharmacologie Cellulaire et Moléculaire, Université Catholique de Louvain, UCL 7370
Avenue E. Mounier 73, Brussels 1200, Belgium
e-mail: marie-paule.mingeot@uclouvain.be

H. Servais
e-mail: helene.servais@wanadoo.be

S. Denamur
e-mail: sophie.j.denamur@uclouvain.be

P. M. Tulkens
e-mail: paul.tulkens@uclouvain.be

A. Ortiz
Fundacion Jimenez Diaz, Unidad de Dialisis, Madrid, Spain
e-mail: AOrtiz@fjd.es

O. Devuyst
Faculty of Medicine, Unité de Néphrologie, Université Catholique de Louvain, Brussels, Belgium
e-mail: Olivier.Devuyst@uclouvain.be

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- κ B	Nuclear factor κ B
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).

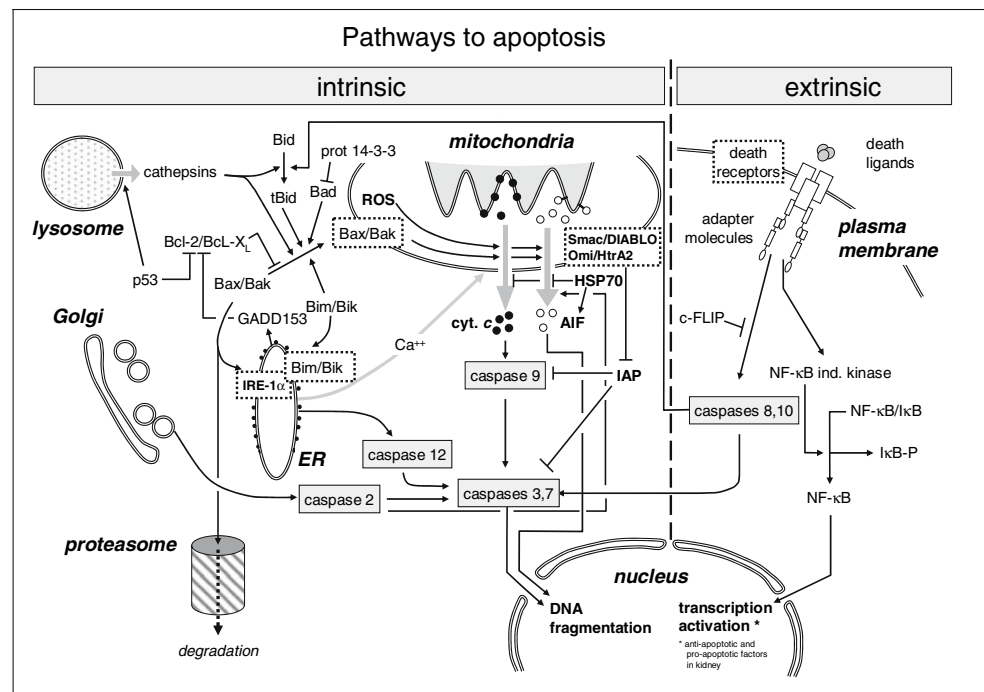
Intrinsic pathway

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]		
			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

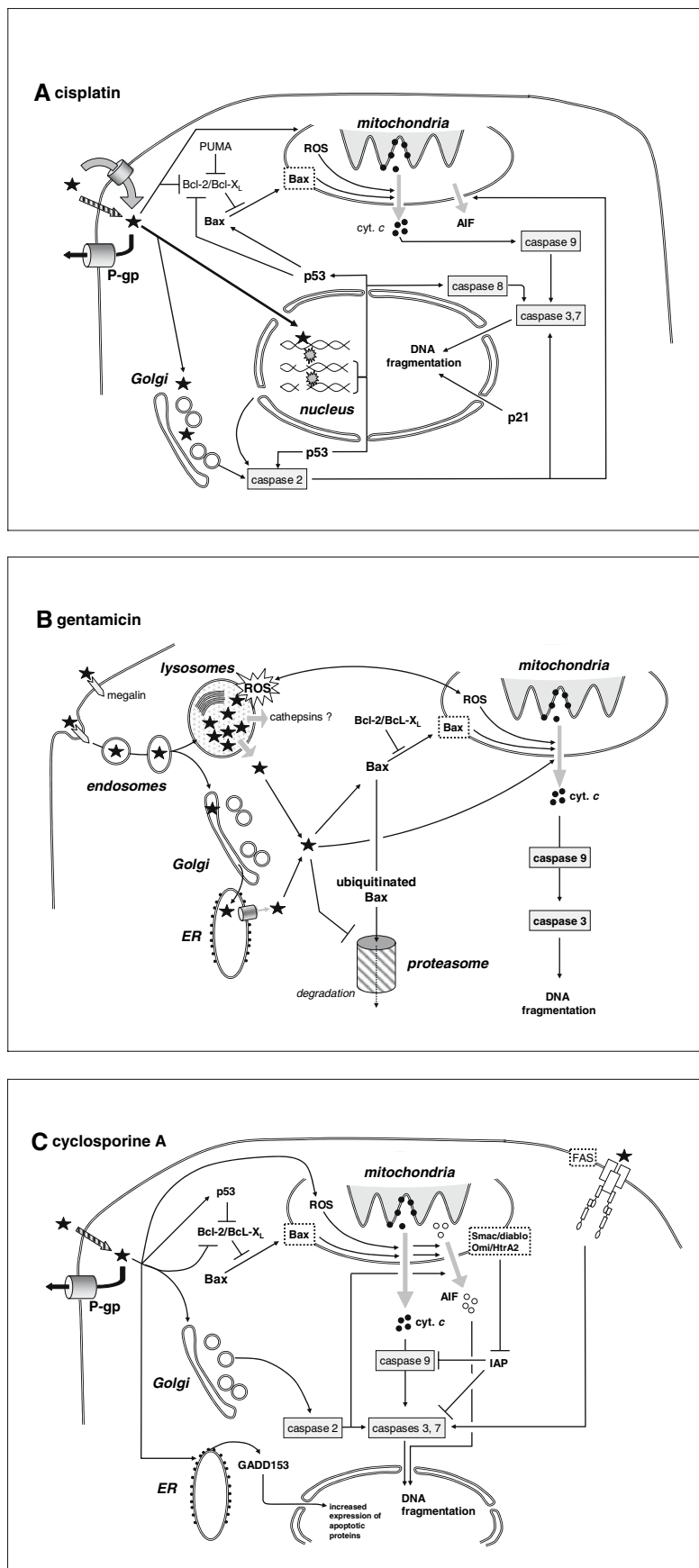


Table 2 Subcellular and molecular pathways involved in drug-induced apoptosis in the renal cells

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

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]	
	Cisplatin	Caspase-12 cleavage	Rabbit renal proximal tubule cells (RPTC)	[213]	
	Cyclosporine A	Increased the activity of ER-iPLA(2)	Murine proximal tubular epithelial	[28]	
	G418	Induction of GADD 153 expression	Rat kidney cells	[212]	
	Tunicamycin	Increase in Ca ²⁺ concentration	GADD 153 ^{-/-} Mice	[45]	
	Gentamicin	Cleavage of m-calpain and procaspase-12	LLC-PK1	[27]	
	Lysosomes	Iodinated contrast media (iobitridol and iohexol)	Decrease of apoptosis in renal proximal tubular epithelium in GADD 153 ^{-/-} Mice	Rats	[214]
			Release of acridine orange		
			Lysosomal permeabilization		
			Prominent lysosomal alteration of the proximal convoluted tubular cells		

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-1 α [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-1 α 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 Ca²⁺ 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-1 β -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- κ B 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- κ B is sequestered in the cytosol by inhibitory proteins known collectively as I κ B. A wide range of apoptotic triggers cause the proteasomal degradation of I- κ B [60], allowing the active NF- κ B 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- κ B is usually anti-apoptotic [61], NF- κ B activation in kidney can also lead to stimulation of apoptosis in renal cells. This is exemplified by TRAIL-mediated NF- κ B 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 endocytosed 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				
<i>Competition with drug binding</i>				
Aminoglycosides	Ca ²⁺ (diet supplementation) Aminoglycosides	Rats Rats	Decrease of blood urinary nitrogen Decrease of renal cortical concentrations	[215] [216]
	Ligands of megalin (lysozyme, aprotinin, cytochrome <i>c</i> , apolipoprotein E3) or chaperone proteins (RAP)	Rats	Reduction of renal cortical concentrations	[121]
		Mouse	Decrease of <i>N</i> -acetyl-glucosaminidase release from lysosomes	[122]
		LLC-PK1		
<i>Impairment of transport</i>				
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]
Cyclosporine A	Competition of uptake by hOCT2	HEK 293	Decrease of cell apoptosis	[119]
	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]
<i>Increase of elimination</i>				
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				
<i>Inhibition of caspases</i>				
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
<i>Action on proteins of Bcl-2 family</i>				
Cisplatin	Minoocycline	Rat kidney proximal tubular cells	Upregulation of Bcl-2 Suppression of Bax accumulation Decrease of outer membrane damage Inhibition of cytochrome <i>c</i> release	[127]
	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 Release of Omi from mitochondria to the cytoplasm	[149]
Cyclosporine A	Bax antisense oligonucleotide	MCT	Degradation of XIAP 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) Pro-apoptotic effect for Bax- and Bak-derived peptides annexin V assay; morphology)	[30]
<i>Action on cellular pathways</i>				
Cisplatin	MAPK/ERK kinase (MEK) inhibitor	Mice	Decreased number of apoptotic cells (morphological studies)	[150]
	Carbon monoxide-releasing molecule acting as a guanylate cyclase activator	Rat	Decreases caspase-3 activity Decrease of apoptosis in tubules at the corticomedulullary junction	[220]
	Prevention of the inhibition of PPAR- α activity	LLC-PK1	Decreased translocation of Bax Increase of Bcl-2 expression	[221]
	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- κ B like parthenolide	Male Wistar Rats	Decreased number of TUNEL-positive cells	[223]
Adriamycin	Prostaacyclins as a suppressor of the activation and translocation of nuclear NF- κ B	NRK-52E	Decreased caspase-3 and 9 activation Inhibition of cytochrome <i>c</i> release Increase of Bcl-2 expression	[203]

Table 3 continued

Drugs responsible for renal apoptosis	Protectant(s)	Model(s)	Effect observed	References
<i>Ligands or proteins specific to receptor-mediated pathway</i>				
Cisplatin	Genetic deletion of either TNF- α or TNFR2	RPT	Reduced cisplatin-induced renal failure, necrosis and apoptosis	[152]
Cyclosporine A	Mineralcorticoid receptor blockers	Rat: subcortical and juxtamedullary cells	Decrease of TUNEL positive cells	[179]
<i>Inhibition of oxidative stress</i>				
Acetaminophen	IH636 grape seed proanthocyanidin extract	Mice	Decreased DNA fragmentation Prevention of renal apoptosis (histology)	[135]
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]
	Dimethylthiourea (hydroxyl radical scavengers)	Male Wistar rats Adult male Wistar Rats	Decreased number of TUNEL positive cells in cortical renal tubules Prevention of the increase of caspase-3 activity	[225, 226] [227]
	Tiron (superoxide scavenger)	Primary cultured rabbit proximal tubule	Decreased cytochrome <i>c</i> release from mitochondria	[206]
	<i>N</i> -Acetylcysteine	Primary cultured rabbit proximal tubule	Reduction of caspase-3 activation	[206]
	Oxathiazolidine derivative (cystein-prodrug)	LLC-PK1	Reduction of caspase-3 activation	[219]
	Trolox (anti-oxidant)	LLC-PK1	Prevention of apoptosis	[228]
	Inhibition of CYP2E1	LLC-PK1	Prevention of apoptosis	[219]
	Sodium dependent glucose transporter (SGLT1) activator	Mice CYP2e1-/- LLC-PK1	Prevention of renal apoptosis (histology) Reduction of peroxynitrite production	[137] [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 <i>Pongamia pinnata</i> (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 Decrease of caspase-3/8 and -9 activities Inhibition of increase in Bcl-x _L expression	[134]
	Chelerythrine	Rats	Prevention of renal apoptosis as evidenced by histological studies	[231]
Radiocontrast Agents	Kallikrein/kinin	Rats	Protection of renal apoptosis	[232]
	Ascorbic acid	Humans	Prevention of renal injury	[233]
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 Inhibition TRAIL-mediated cytochrome <i>c</i> release from the mitochondria and caspase-3-like activation	[235]
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]
Cyclosporine A		Mouse inner medullary collecting duct	Rescue of cyclosporine A-induced tubular injury	[236]
		Rats transfected with HGF gene	Inhibition of tubular cell apoptosis	[159]
		Human proximal tubular cell	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 than 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 NH₂-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 P₄₅₀ 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 downregulates 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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