ANTIOXIDANTS & REDOX SIGNALING
Volume 4, Number 6, 2002

Forum Review

Apoptotic Pathways of Oxidative Damage to Renal Tubular Epithelial Cells

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ABSTRACT

Toxic renal failure induced by gentamicin, glycerol, or cisplatin, as well as ischemic renal failure *in vivo* and hypoxia/reoxygenation of tubular epithelial cells *in vitro,* induces the production of reactive oxygen metabolites (ROM). Generation of ROM is responsible for the induction of tubular epithelial cell death, which is mediated by caspases and/or endonucleases. Scavenging of ROM protects tubular epithelium from caspase and endonuclease activation and from cell death. Thus, the inhibition of ROM production combined with the pharmacological control of caspase and endonuclease pathways may provide future modalities in the prevention or treatment of acute renal failure in humans. *Antioxid. Redox Signal.* 4, 915–924.

INTRODUCTION

THE FIELD OF REACTIVE OXYGEN METABOLITES (ROM) or oxidants, more loosely referred to as "free radicals," has reached a stage where television and magazine ads tout the virtues of antioxidant vitamins. The notion that ROM may be important in inflammation was initiated by a publication in 1969, in which McCord and Fridovich described an enzyme, superoxide dismutase, which scavenges superoxide anion (46). McCord reasoned that as phagocytizing neutrophils (the effector cells of the acute inflammatory response) release large amounts of superoxide extracellularly and superoxide dismutase (an enzyme that scavenges superoxide) possesses antiinflammatory activity, the superoxide anion and other oxygen metabolites may be important chemical mediators of the inflammatory process (44). This hypothesis has received considerable support from a large number of studies over the last decade that indicate that partially reduced oxygen metabolites are important mediators of ischemic, toxic, and immune-mediated tissue injury (31, 46).

In this review, we summarize the current evidence for a role of ROM in toxic acute renal failure including gentamicin-, glycerol-, cisplatin-induced acute renal failure. Then we will discuss the role of endonucleases in deoxyribonucleic acid (DNA) fragmentation mediated by ROM, and the role of caspases and ceramide contributing to the oxidant injury in the kidney.

Oxygen normally accepts four electrons and is converted directly to water. However, partial reduction of oxygen can and does occur in biological systems. Thus, the sequential reduction of oxygen along the univalent pathway leads to the generation of superoxide anion, hydrogen peroxide, hydroxyl radical, and water (24, 31).

Superoxide and hydrogen peroxide appear to be the primary species generated. These species may then play a role in the generation of additional and more reactive oxidants, including the highly reactive hydroxyl radical (or a related highly oxidizing species) in which iron salts play a catalytic role in a reaction, commonly referred to as the metal-catalyzed Haber–Weiss reaction (31).

$$Fe^{3+} + O_2^- \rightarrow Fe^{2+} + O_2$$

$$\frac{Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-}{O_2^- + H_2O_2 \rightarrow O_2 + \cdot OH + OH^-}$$

Additional ROM can be formed as a result of the metabolism of hydrogen peroxide by neutrophil-derived myeloperoxidase (the enzyme responsible for the green color of pus) to produce highly reactive toxic products, including hypochlorous

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acid. These oxygen metabolites, including the free radical species superoxide and hydroxyl radical, and other metabolites, such as hydrogen peroxide and hypohalous acids, are often collectively referred to as reactive oxygen metabolites.

ROLE OF ROM IN ACUTE RENAL FAILURE

Role of ROM in gentamicin nephrotoxicity

A major complication of the use of aminoglycoside antibiotics, including gentamicin, which are widely used in the treatment of gram-negative infections, is nephrotoxicity, which accounts for 10–15% of all cases of acute renal failure (34). The precise mechanism(s) of gentamicin nephrotoxicity remains unknown. *In vitro* and *in vivo* studies indicate enhanced generation of hydrogen peroxide and release of iron in response to gentamicin. Most, if not all, of the hydrogen peroxide generated by mitochondria is derived from the dismutation of superoxide. Thus, the enhanced generation of hydrogen peroxide by gentamicin suggests that superoxide anion production is also increased. Superoxide and hydrogen peroxide may interact (with trace metals such as iron as the redox agent) to generate highly reactive and unstable oxidizing species, including the hydroxyl radical. Several studies have, in fact, shown that agents that enhance the generation of hydrogen peroxide and superoxide anion by mitochondria also enhance the generation of hydroxyl radical. We have demonstrated that hydroxyl radical scavengers and iron chelators provide a marked protective effect on renal function in gentamicin-induced acute renal failure in rats (51). In addition, histological evidence of damage was markedly reduced by the interventional agents. Recent studies from other laboratories have provided support for these observations. Administration of superoxide dismutase or an oxidant scavenger, dimethylthiourea, provided a marked protection against gentamicin-induced impairment of renal function and lipid peroxidation, and dimethylthiourea attenuated the tubular damage (51). In contrast, it was reported that despite amelioration of gentamicin-induced lipid peroxidation by the treatment of an antioxidant, diphenylphenylenediamine, it failed to prevent nephrotoxicity (60). However, it was also demonstrated that coadministration of antioxidants, vitamin E and selenium, is protective against gentamicin-induced nephrotoxicity (1). It is not clear why the contradictory results are obtained; however, one explanation is that it may be due to the difference in the mechanisms of the protective effect of antioxidants. Additional support for a role of iron-catalyzed free radical generation has been provided by demonstrating that gentamicin-induced generation of hydroxyl radicals is reduced by iron chelators *in vitro* (83) and iron supplementation enhances gentamicin nephrotoxicity *in vivo* (11, 39). Taken together, it appears that ROM are one of the mediators responsible for gentamicin nephrotoxicity.

Role of ROM in glycerol-induced acute renal failure

During the Battle of Britain, Bywaters and Beall (15) described the first causative association of acute renal failure with skeletal muscle injury with the release of muscle cell contents, including myoglobin, into plasma (rhabdomyolysis). Since then, the spectrum of etiologies for rhabdomyolysis, myoglobinuria, and renal failure has been markedly expanded with the recognition of both traumatic and, more recently, nontraumatic causes (25, 26, 42). The most widely used model of myoglobinuric acute renal failure is produced by subcutaneous or intramuscular injection of hypertonic glycerol (33). We have demonstrated enhanced generation of hydrogen peroxide in glycerol-induced acute renal failure (27) utilizing the method described for demonstrating enhanced *in vivo* generation of hydrogen peroxide in response to gentamicin. In a recent study, Zager (86) has provided evidence for mitochondria as a critical site of heme-induced free radical formation (87). When heme-laden proximal tubular segments were exposed to mitochondrial respiratory chain inhibitors, there was a marked alteration in lipid peroxidation: blockade at site 2 or site 3 prevented heme-induced lipid peroxidation, whereas blockade at site 1 increased oxidative damage.

The recognition that hydrogen peroxide is produced in excessive amounts in this model motivated the examination of the potential efficacy of pyruvate, an α-ketoacid (63). A property shared by a wide range of α-ketoacids is the ability of these metabolites to scavenge hydrogen peroxide through a nonenzymatic oxidative decarboxylation reaction (14). The administration of pyruvate, following the intramuscular injection of glycerol, improved renal function as measured by serum creatinine determinations accompanied by a marked reduction in structural injury (63). A property of pyruvate that perhaps contributes to its protective effect is its facile distribution across plasma and mitochondrial membranes (30, 49). This is an attribute that delivers pyruvate widely within the intracellular compartment and to subcellular sites at which potentially damaging peroxides are produced.

We have also examined the effect of hydroxyl radical scavengers and iron chelators in glycerol-induced acute renal failure in rats (66). Dimethylthiourea, a hydroxyl radical scavenger, provided marked protection against glycerol-induced acute renal failure. In contrast to the effect of dimethylthiourea, urea (which is not a hydroxyl radical scavenger and served as a control) failed to provide any protection. A second hydroxyl radical scavenger, sodium benzoate, and an iron chelator, deferoxamine, had a similar protective effect on renal function. The interventional agents were also associated with a marked reduction in histological evidence of renal damage. Paller has also demonstrated that deferoxamine treatment was protective in three models of myoglobinuric renal injury, namely, hemoglobin-induced nephrotoxicity, glycerol-induced acute renal failure, and a combined renal ischemia hemoglobin insult (56). Similarly, Zager in his studies has demonstrated the protective effect of an iron chelator in myohemoglobinuric injury (85). Taken together, the histological and functional protective effect of the hydroxyl radical scavengers and an iron chelator implicates a role for the hydroxyl radical in glycerol-induced acute renal failure.

Role of ROM in cisplatin-induced nephrotoxicity

Cisplatin is a widely used antineoplastic agent, which has nephrotoxicity as a major side effect. The underlying mecha-

nism of this nephrotoxicity is still not well known. We have examined the catalytic iron content and the effect of iron chelators in an *in vitro* model of cisplatin-induced cytotoxicity in LLC-PK_1 cells [renal tubular epithelial (RTE) cells] and in an *in vivo* model of cisplatin-induced acute renal failure in rats (7). Exposure of LLC-PK_1 cells to cisplatin resulted in a significant increase in bleomycin-detectable iron (iron capable of catalyzing free radical reactions) released into the medium. Concurrent incubation of LLC-PK_1 cells with iron chelators, including deferoxamine and 1,10-phenanthroline significantly attenuated cisplatin-induced cytotoxicity as measured by lactate dehydrogenase (LDH) release. Bleomycin-detectable iron content was also markedly increased in the kidney of rats treated with cisplatin. Similarly, the administration of deferoxamine in rats provided marked functional (as measured by blood urea nitrogen and creatinine) and histological protection against cisplatin-induced acute renal failure. In a separate study, we examined the role of the hydroxyl radical in cisplatin-induced nephrotoxicity. Incubation of LLC-PK_1 cells with cisplatin caused an increase in hydroxyl radical formation. Hydroxyl radical scavengers, dimethyl sulfoxide, mannitol, and benzoic acid, significantly reduced cisplatin-induced cytotoxicity, and treatment with dimethyl sulfoxide or dimethylthiourea provided significant protection against cisplatin-induced acute renal failure. Taken together, our data strongly support a critical role for iron in mediating tissue injury via hydroxyl radical (or a similar oxidant) in this model of nephrotoxicity.

MECHANISMS OF RTE CELL INJURY

DNA fragmentation associated with oxidative damage to the kidney

There is ample evidence that DNA damage is an early event in response to a wide variety of insults. Much of the evidence for the role of cell death mechanisms in RTE cell injury relates to endonuclease activation resulting in DNA fragmentation (10, 35, 37, 53, 74, 75). Schumer *et al.* (65) were among the first to describe DNA fragmentation in the kidney cortex after reperfusion. Nogae *et al.* (53) reported the 200 bp-fold DNA fragmentation pattern after subjecting kidneys to ischemia/reperfusion. Iwata *et al.* (35) have shown 200 bp-ladder formation in postischemic rat kidneys using a terminal deoxynucleotide transferase end-labeling assay. This DNA fragmentation was accompanied by morphological features of necrosis rather than apoptosis. Similarly, isolated perfused rat kidneys subjected to hypoxia developed DNA strand breaks in tubular epithelium (10).

Cell death by both apoptosis and necrosis is associated with DNA strand breaks, although in apoptosis the nuclear envelope is not damaged (3). In various tissues, chromosomal DNA degradation during apoptosis is linked to an unknown deoxyribonuclease (DNase) I-like alkaline Ca- or Ca/Mg-dependent Zn-inhibitable endonuclease (50, 81, 82). This endonuclease generates internucleosomal 3′OH/5′P DNA strand breaks, which are visualized at later stages as 200-bp ladder in agarose (81, 82). Importantly, necrosis often shares some features of apoptosis, and the 200-bp ladder is one of them (35, 75). The initial suggestion that necrosis produces a "smear" instead of the ladder most likely depends on the integrity of the nuclear membrane and the activity of cellular proteinases sufficient to remove histones from the DNA. Although DNase II-type endonucleases (acidic, cation-independent, generating 3′P/5′OH ends) of lysosomal origin were described in association with necrosis (79), it is unlikely that any endonuclease can produce a pattern different from the 200-bp ladder until the nucleosomal structure of chromatin is destroyed.

Direct and indirect DNA strand breaks induced by oxidative stress

DNA strand breaks generated during oxidative stress are originated either by nonenzymatic direct oxidative damage (16) or by the cleavage produced by endonucleases (82). It has been shown that in different tissues as well as in the kidney, ROM destroy the deoxyribose skeleton of the DNA generating oligonucleotides with characteristic 3′-phosphoglyconane termini (12, 16). These DNA strand breaks cannot be immediately repaired by ligation without prior conversion to 3′OH ends, because enzymes for such reaction are not known. Considering the small sizes of the ROM molecules, the length of DNA fragments should not reflect the chromatin structure. Therefore, all of the observed 200-bp ladder DNA fragmentation is a product of DNases/endonucleases. Double-strand DNA breaks generated by an endogenous DNase/endonuclease are considered a "point of no return" when cell death becomes irreversible. The endonuclease activation in hypoxia/reoxygenation injury to the kidney does not necessarily lead to the morphological features of apoptosis, such as chromatin condensation (75).

An increase of direct oxidative breaks is observed within minutes after a hydrogen peroxide treatment of LLC-PK_1 cells; however, the ladder-type DNA fragmentation follows in several hours (74). A similar sequence of events was observed in ischemia/reperfusion (I/R) injury to rat kidney (9). An initial spike of oxidative DNA damage measured by 8-hydroxyguanosine observed in ischemia was followed by an increase of DNase I-like endonuclease activity in kidney cortex, which takes 1–24 h. Despite the fact that endonuclease activation is secondary to the initial oxidative stress, it seems to be directly responsible for the cell death. We have shown that endonuclease inhibitors, aurintricarboxylic acid, Evans blue, and zinc ion prevented DNA fragmentation and cell death of LLC-PK_1 cells induced by hydrogen peroxide or chemical hypoxia (28, 74).

Approximately 40 double-strand DNA breaks per cell has been shown to be lethal (58). Beyond this level, the repair of DNA breaks is no longer effective. The sensitivity of the method used to measure DNA strand breaks determines to a great extent the time point when the DNA fragmentation can be registered. Therefore, the notion of endonuclease activation occurring much later than the direct oxidative stress may not be always correct. The 200-bp ladder, which is commonly used because of its simplicity, actually measures very late cell death events. Clearly, the chromatin does not need to be cut to the 200-bp fragments to induce cell death. In addition, the activity of endonucleases in some cells is very low, and internucleosomal DNA fragmentation cannot be easily reached. This led some investigators to a conclusion that endonucleases are

not involved in renal cell death induced by hypoxia (54). Other methods, which are aimed to quantify rare DNA breaks (pulse-field electrophoresis, random oligonucleotide-primed synthesis assay) or to detect DNA fragmentation in individual cells [TdT-mediated dUTP nick-end labeling (TUNEL)], provide more accurate timing of DNA fragmentation. In our experience, these assays detect DNA breaks at 1–3 h after the insult, whereas the 200-bp ladder appears at 8–24 h, depending on the model (9, 74).

Renal endonucleases

Limited information is available regarding the endonuclease(s) responsible for the DNA fragmentation in the kidney. Our studies showed the presence of two major endonucleases in kidneys (rat, mouse, pig) and kidney cells (LLC-PK_1, NRK-52E), 15-kDa endonuclease and 30–34-kDa DNase I-like endonuclease (9, 28, 74, 75). The latter is mainly a cytoplasmic enzyme, whereas the 15-kDa endonuclease is located in the nuclei. The proportion of these endonucleases varies in different species and cell lines. The activity of 30-kDa endonuclease is increased during I/R in rat kidney (9). This enzyme was similar to a DNase I by its biochemical characteristics. Hypoxia/reoxygenation resulted in an increase in up-regulation of the 15-kDa endonuclease, which preceded nuclear DNA fragmentation and cell death (75). *In vitro*, this endonuclease was Ca-dependent and was not inhibited by zinc. When applied to cultured cells, zinc sulfate provided effective protection against hydrogen peroxide-induced DNA fragmentation and cell death (74), and partially protected against antimycin A-induced cell death (28). Taken together these data provide strong evidence for a role of one or several endonucleases in DNA damage and cell death in hypoxia/reoxygenation injury to the kidney. As shown in some experimental models, two or more endonucleases present in the same tissue can participate in cell death (17).

Among other endonucleases available for DNA fragmentation in the kidney, DNase I, DNase γ, DNase II, and caspase-activated DNase have been described (22, 57, 79). DNase I is found in all studied species and tissues (72). It is expressed principally in tissues of the digestive system, though the specific activity of the enzyme varies (43). In digestive tissues (intestine, pancreas, salivary glands), it is a secretory enzyme intended to hydrolyze DNA in the alimentary tract. In nondigestive tissues (including kidney), the role of DNase I is not known. Among various organs and tissues, the kidney has one of the highest levels of DNase I activity as measured using DNA-substrate gel electrophoresis (43, 72). Little information is available about how DNase I can be regulated *in vivo*. Some DNase I isoforms can be generated by posttranslational modification, namely mannose-type glycosylation of the protein (43). A caspase-activated deoxyribonuclease (CAD) of 40 kDa has been identified by Nagata's group in the cytoplasmic fraction of mouse lymphoma cells (22). CAD is the most well documented example of an apoptotic endonuclease. This enzyme is present in mouse and human kidney, whereas some other tissues were found to be CAD-negative (48).

Activation of endonucleases by ROM

The activation of endogenous endonucleases in response to oxidative stress was known since the work of Skalka and Matyasova in the 1960s (69). The exact sequence of events leading to the activation of endonuclease by oxidants is not clear. Our data indicated that different reactive oxygen species contribute to the activation of endonuclease and the enzymatic DNA damage induced by chemical hypoxic injury to RTE cells (29). Significant protection against DNA strand breaks induced by chemical hypoxia was provided by superoxide dismutase, a scavenger of the superoxide radical, by pyruvate, a scavenger of hydrogen peroxide, by hydroxyl radical scavengers, such as dimethylthiourea, salicylate, and sodium benzoate, and by metal chelators, deferoxamine and 1,10-phenanthroline. The association of endonuclease activation with different ROM can be suggestive of the absence of a direct link between the type of ROM species and the type of endonuclease. Various ROM can lead to activation of 15-kDa endonuclease, 30-kDa DNase I-like endonuclease, and possibly other endonucleases.

Do we need to prevent DNA fragmentation in the kidney?

The activation of endonucleases is both an early and late event in oxidant injury to kidney cells. Although cell death without detectable DNA fragmentation has been described (82), multiple studies by several groups have shown a direct link between endonuclease-generated DNA breaks and subsequent cell death in different systems (22, 41, 50, 57, 90). We have demonstrated that endonuclease inhibitors prevented DNA damage and cell death in oxidant or hypoxic injury to LLC-PK_1 cells (7, 28) and in hypoxic injury to isolated proximal tubules (75) (Fig. 1). There is also direct evidence that overexpression of DNase I (57), DNase II (41), and CAD (22) cause DNA fragmentation and irreversible cell death. Acting alone, each of these DNases is capable of causing cell death. On the other hand, a significant portion of DNA breaks oc-

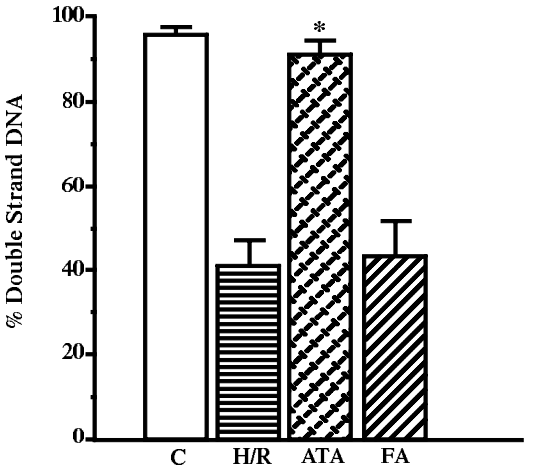

FIG. 1. Effect of endonuclease inhibitor, aurintricarboxylic acid (ATA), on hypoxia/reoxygenation (H/R)-induced DNA strand breaks in isolated rat kidney proximal tubules. Tubules were incubated with 100 μ*M* ATA or its inactive analogue, fuchsin acid (FA; 100 μ*M*). The residual double-stranded DNA was measured by the alkaline unwinding assay. Results are means ± SE ($n = 4$). *$p < 0.005$ compared with hypoxia (30 min) and reoxygenation (30 min) alone.

curs after cell death. These DNA breaks are very important in the "clean-up" of the debris of dead cells. Failure of DNA fragmentation mechanisms responsible for this process may lead to a pathological condition due to accumulation of extracellular DNA. Recently produced DNase I-deficient mice develop a lupus-like syndrome characterized by the presence of antinuclear antibody and glomerulonephritis (52).

The prevention of endonuclease activation and DNA fragmentation may be possible if it targeted inhibiting initial sublethal DNA breaks. Importantly, any manipulations of the endonuclease activity and DNA breaks near the "point of no return" or beyond may lead to abortive apoptosis (abortosis), mutations, and cancer (59). From the perspective of anti-DNase therapy, important factors are the spectrum of endonucleases in the tissue and the mechanisms of their regulation. Investigators will need to know which of the endonucleases (if any) are induced through a short pathway and which are regulated via multistep mechanisms. In each of these cases, the most upstream event should be targeted. If the activation of endonuclease is the immediate response to the oxidative damage (for example, due to direct oxidative modification of the endonuclease molecule), such endonuclease can be suppressed directly. Obviously, additional studies are necessary to ensure that reversal of cell death at this point is not harmful to the cells.

Caspases and cell death in hypoxia/reoxygenation injury to RTE

Caspases are a family of intracellular cysteine proteases that play an essential role in the execution phase of apoptosis, upstream to endonucleases. Currently, there is limited information on the role of caspases in hypoxic renal tubular cell injury. Exposure of hypoxia to freshly isolated RTE cells resulted in caspase activation, cell membrane damage (21), and necrotic cell death. The pan-caspase inhibitor attenuated the hypoxia-induced increase in caspase activity in RTE and provided protection against hypoxia-induced cell membrane damage, as determined by LDH release (21). In our previous studies, we have demonstrated that chemical hypoxia with antimycin A results in increased caspase activity that precedes DNA damage and cell death. The caspase inhibitors prevented hypoxia-induced DNA fragmentation as determined by agarose gel electrophoresis and by *in situ* labeling of cell nuclei by the TUNEL method (36). Partial ATP depletion of MDCK cells by antimycin A was also shown to result in apoptosis with marked increase in activation of caspase-8, and the caspase inhibitors provided marked protection against antimycin A-induced cell death (23). In a related study, activation of caspase-3 during hypoxia or ATP depletion was accompanied by the translocation of Bcl-2 family member, bax, from the cytosol to the mitochondria and the release of cytochrome *c* from the mitochondria to the cytosol (62). These studies indicate that caspases may be involved in both apoptosis and necrosis in RTE cells.

Caspases and cell death in ischemic acute renal failure

Studies from several laboratories have provided evidence that both apoptosis and necrosis of RTE cells occur in experimental models of acute renal failure. The initial evidence of apoptosis in ischemic acute renal failure was observed by Schumer *et al.* (65) in rat kidney cortex 12 h after reperfusion injury. Further studies have documented DNA laddering and/or morphological changes of apoptosis in I/R injury to kidneys (8, 53, 68). A longer period of ischemia induces both apoptosis and necrosis (53, 68), whereas a shorter period of ischemia induces apoptosis without any evidence of necrosis. Although caspases have been implicated in cell death, there is limited information on the specific role of caspases in ischemic injury. We have examined the gene expression of caspases in kidneys subjected to I/R injury (37). The mRNA levels of caspase-2 and caspase-6 showed a marked transient increase during 40 min of ischemia, which then returned to basal levels during reperfusion. On the other hand, caspase-1 and caspase-3 mRNA levels were significantly up-regulated during reperfusion. The proforms of caspase-1 and caspase-3 were cleaved to their active forms during reperfusion, indicating activation of these enzymes (37). These data indicate the differential regulation of caspases and their role for apoptosis in ischemic acute renal failure. Caspase-3 activity was significantly increased in the rat and murine models of renal I/R injury (67). The administration of pan-caspase inhibitor, Z-VAD-FMK, at the time of reperfusion significantly prevented caspase-1 and caspase-3 activities, and provided marked protection not only against renal tubular apoptosis and subsequent inflammation, but also ischemic acute renal failure (19). These results in renal ischemic injury seem consistent with the recent study performed on ischemic injury to gerbil forebrain (40) and rat brain (4). A recent study on global forebrain ischemia has reported increased mRNA and protein expression of caspase-1 at 48 h after ischemia in gerbils (13). Increased induction of caspase-3 mRNA at 16 h through 24 h after ischemic injury has also been reported in rat brain after permanent occlusion of the middle cerebral artery (4). The specific role of proinflammatory caspase-1 has recently been examined in ischemic acute renal failure. Caspase-1 is involved in the proteolytic cleavage of the precursor forms of proinflammatory cytokines interleukin-1β (IL-1β) and IL-18 that result in the formation of active forms of mature cytokines. As caspase-1-mediated formation of active IL-1β (32) and IL-18 (20) are associated with inflammation in renal I/R, caspase-1 may play an important role in I/R injury. Thus far, two recent studies have investigated the role of caspase-1 in I/R injury using caspase-$1^{-/-}$ mice, but the results have remained inconsistent. One study reported that caspase-$1^{-/-}$ mice provided significant protection against I/R as reflected by renal function and renal histology (47), whereas the other study demonstrated that caspase-$1^{-/-}$ mice did not provide protection against I/R as revealed by renal function with no change in blood urea nitrogen and serum creatinine (18). Thus, more studies are required to demonstrate the definitive contribution of caspase-dependent and caspase-independent formation of inflammatory products for the induction of inflammation and apoptosis in ischemic acute renal failure. A recent study has demonstrated that caspase-3 activation during I/R injury may be involved in the down-regulation of calpastatin, an inhibitor of calpain (67), indicating a role of caspases for calpain activation during renal injury.

Caspases and cell death in toxic acute renal failure

Chemotherapeutic agents (2), antibiotics, radiocontrast substances, and other nephrotoxins, including some occupational and environmental agents (5), can induce renal tubular injury. Among the chemotherapeutic agents that cause nephrotoxicity, the effect of cisplatin on proximal tubular epithelial cell injury has been extensively studied. The primary targets of cisplatin in the kidney are the proximal tubular epithelial cells, where it accumulates and promotes the damage of these cells (61). The cellular and molecular mechanisms responsible for drug-induced nephrotoxicity to RTE cells are not well understood. Cisplatin has been shown to induce cell death in RTE cells (55, 70). Caspase-3 is activated in renal proximal tubular cells by cisplatin treatment, suggesting that cisplatin-induced cell death is mediated by caspases. Our data demonstrated that cisplatin induces selective and differential activation of caspases, including executioner caspase-3 and initiator caspase-8 and caspase-9, but not proinflammatory caspase-1 (38). The selective activation of these caspases was markedly inhibited by their respective peptide inhibitors, suggesting that these caspases may play an important role in cisplatin-induced injury to RTE cells (Fig. 2). DEVD-CHO or LEHD-CHO, inhibitors of caspase-3 and caspase-9, respectively, provided marked protection against cisplatin-induced cell death and partial protection against DNA damage in LLC-PK_1 cells as revealed by an alkaline unwinding assay and by agarose gel electrophoresis (38). The specific role of caspase-3 and its more direct involvement in cisplatin-induced injury has come from studies utilizing the baculovirus protein p35, which is a potent inhibitor of caspase-3 (38). Overexpression of p35 blocks the induction of apoptosis in insect and mammalian cells. Thus, a stably transfected LLC-PK_1 cell line developed to overexpress p35 was capable of providing protection against cisplatin-induced injury, indicating that cisplatin injury involves the participation of caspases (38). Overexpression of crmA, a cowpox viral gene known to inhibit caspase-8, also provided protection against cisplatin-induced apoptosis in mouse proximal tubular cells (71). Thus, cisplatin-induced activation of caspase-8 and caspase-9 in renal proximal tubules indicates that both receptor and mitochondrial pathways participate in the activation process.

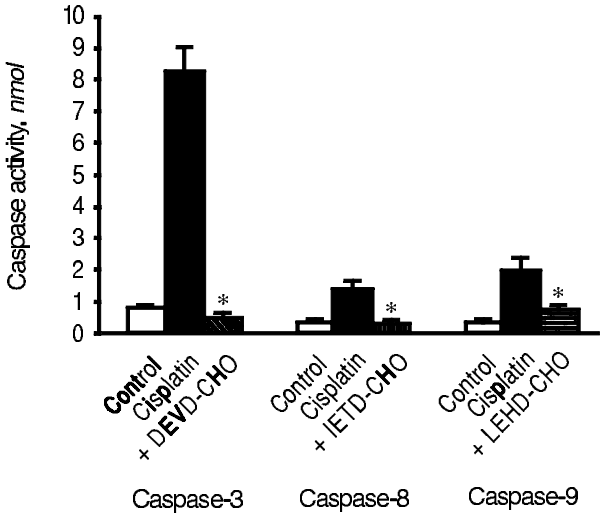

FIG. 2. Effect of caspase inhibitors on cisplatin-induced caspase activation. Cells were treated with 50 μmol/L cisplatin for 20 h in the presence or absence of caspase inhibitors (50 μmol/L): DEVD-CHO for caspase 3, IETD-CHO for caspase 8, and LEHD-CHO for caspase 9. Results are means ± SE (n = 4). *p < 0.001 compared with cisplatin-treated controls.

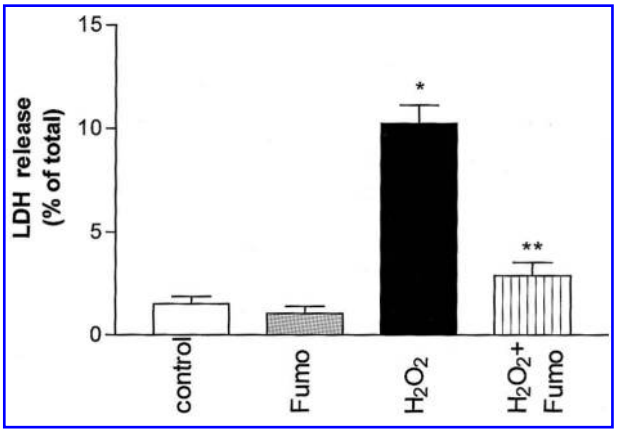

FIG. 3. Effect of ceramide synthase inhibitor fumonisin B1 on H_2O_2-induced cell death. Cells were preincubated with fumonisin B1 (50 μ*M*) for 30 min, and then exposed to 1 m*M* H_2O_2 for 60 min. Cell viability was measured by LDH release (% of total LDH). Results are means ± SE (n = 8–13). *p < 0.0001 compared with control cells; **p < 0.04 compared with cells exposed to H_2O_2 alone.

Ceramide

In addition to caspases, ceramide has been recently recognized as another important modulator of endonuclease-mediated DNA damage and apoptosis. In different models, caspase-8 inhibition abrogated ceramide formation (18, 73) and reduced ceramide-induced cell death (18, 80). Studies showed that some ROM, singlet oxygen and H_2O_2, induce an increase of caspase-8 (89, 91). Blockage of caspase-8 by Z-IETD-fmk reduced ROM generation by mitochondria (84) and inhibited endonuclease-mediated DNA fragmentation (92).

We have shown an important role of ceramide in H_2O_2- and hypoxia/reoxygenation-induced DNA damage and necrotic cell death in RTE cells (76, 77). Hydrogen peroxide increases ceramide synthase activation and ceramide generation without any significant change in sphingomyelin content and sphingomyelinase activity (74). Inhibition of ceramide synthase using fumonisin B1 prevented H_2O_2-induced DNA damage and cell death in NRK-52E cells (Fig. 3). These data suggest that the major enzyme responsible for ceramide generation in oxidant injury is ceramide synthase rather than sphingomyelinases. In contrast, Zager *et al.* (88, 89) revealed increased ceramide level during reperfusion of ischemic mouse kidney and in hypoxic RTE cells accompanied by a decrease in sphingomyelinase activity. We have shown that subjecting RTE cells to hypoxia/reoxygenation or subjecting rat kidneys to I/R results in increased ceramide generation and ceramide synthase activity without significant change in

sphingomyelinase activity and sphingomyelin content (76). These data indicate that the pathway of ceramide generation varies with cell types, and that the ceramide synthase-dependent pathway is of major importance in hypoxic or ischemic renal injury. Currently, it is not known how ceramide synthase, and hence the enhanced generation of ceramide, is regulated by the mediators in oxidant and hypoxia/reoxygenation renal injury.

CONCLUSION

Traditionally, ischemic and toxic acute renal failure have been considered to lead to the necrotic form of cell death. The studies described above indicate that the apoptotic mode of cell death is also very important in RTE cell injury. The pathway that is followed by the cell is dependent on both the nature and severity of insults, evolving from the apoptotic to the necrotic form of cell death. Many recent clinical trials of acute renal failure have not shown beneficial effects. Studies that implicate apoptotic pathways suggest that targeting mild to moderate renal failure toward endonucleases and pathways that regulate them may provide new therapeutic opportunities of acute renal failure.

ABBREVIATIONS

CAD, caspase-activated deoxyribonuclease; DNA, deoxyribonucleic acid; DNase, deoxyribonuclease; IL, interleukin; I/R, ischemia/reperfusion; LDH, lactate dehydrogenase; ROM, reactive oxygen metabolites; RTE, renal tubular epithelium; TUNEL, TdT-mediated dUTP nick-end labeling.

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Received for publication January 18, 2002; accepted May 6, 2002.

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