Molecular Response to Cytotoxic Injury: Role of Inflammation, MAP Kinases, and Endoplasmic Reticulum Stress Response

By Joseph V. Bonventre

Nephrotoxicants have varied direct and indirect effects on the vasculature, tubules, and interstitium of the kidney. In most cases the molecular components of the toxic insult are poorly understood. In this review some common themes of injury, repair, and adaptive protective responses that represent characteristic responses of the cells and kidney tissue that transcend the specifics of a particular toxin are presented. Particular attention is paid to the vascular and inflammatory aspects of nephrotoxicity as well as the activation of the MAP kinase families and the endoplasmic reticulum stress response by the tubular epithelial cell.

NEPHROTOXICANTS CAN HAVE diverse effects on the kidney, which are manifest in a variety of clinical syndromes. Some, such as nonsteroidal anti-inflammatory agents or cyclosporin, can alter vascular tone or intrarenal hemodynamics and result in prerenal azotemia, which may progress to ischemia. Shiga toxin has a direct effect on the endothelial cell. A number of drugs, such as the penicillins, sulfonamides, and cimetidine, cause a tubulointerstitial nephritis. Intrarenal obstruction can occur when crystalline deposits form in the renal tubule in response to acyclovir or high-dose methotrexate therapy. Toxicants, such as aminoglycosides, amphotericin B, cisplatin, and heavy metals, can directly damage the tubule epithelial cell. But even when the injury is primarily to the epithelial cell there is associated inflammation with important involvement of the vasculature. The glomerulus can be the target of the toxin, resulting in proteinuria and in some cases nephrotic syndrome. With milder forms of injury the clinical consequences may be functional only, such as the nephrogenic diabetes insipidus that occurs with lithium or democlocycline therapy. This is the case with gold, penicillamine, or heroin. Nephrotoxicity, therefore, cannot be understood if looked on narrowly as a problem involving a toxin and the cell primarily affected by it because the fate of the tubular or glomerular epithelial cell is very dependent on the response of the surrounding vasculature and in many cases infiltrating cells that are part of an inflammatory response. Major consequences of nephrotoxicity are the resultant acute renal failure and in some cases chronic renal failure, which results from the exposure to the toxicant. The molecular response of the renal epithelial cell can be adaptive but also may be maladaptive under certain conditions. The literature of nephrotoxicity is not very amenable to integration because most often specific features of the pathophysiology of a particular toxicant are presented and generally are not related to other toxicants. This brief review focuses on common features of injury to the kidney that, in many cases, characterize both toxicant-induced injury as well as injury associated with ischemia/reperfusion, which is studied much more extensively.

In animals, exposure to toxicants or ischemia often results in rapid loss of epithelial cell cytoskeletal integrity and cell polarity. The proximal tubular brush border is disrupted.[1] There is abnormal localization of adhesion molecules and other membrane proteins such as Na^+K^+adenosine triphosphatase, blebbing of apical membranes, cellular and mitochondrial swelling, and pyknosis of nuclei and apoptosis. With advanced injury, tubular epithelial cells detach from the basement membrane and contribute to intraluminal aggregations of cells, proteins, and glycoproteins such as fibronectin, resulting in obstruction.[2,3] These changes have been studied most extensively with ischemia/reperfusion. If the toxicant or ischemic influence is removed in a time frame within which permanent changes to the kidney have not occurred, the kidney has the ability to repair and restore its functional integrity. When the kidney recovers from an acute insult the surviving epithelial cells dedifferentiate and spread over the base-

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ment membrane, which has been partially denuded by loss of epithelial cells.[1] Cell proliferation then occurs to restore cell number,[4] followed by differentiation that results in restoration of the functional integrity of the nephron.[2,5,6]

HEMODYNAMIC FACTORS

A number of renal toxins have vasoconstrictor influences. The calcineurin inhibitors, cyclosporine and tacrolimus, that have been so effective in reduction of allograft rejection, have significant nephrotoxic effects of which vascular effects are believed to be a central component. These agents have been associated with renal and systemic vasoconstriction, increased release of endothelin-1, decreased production of nitric oxide, and increased expression of transforming growth factor (TGF-β).[7]

Cyclosporine and mycophenolate mofetil induce a dysfunction of the vasorelaxing properties of the endothelium that may lead to a decrease in the protective effects of nitric oxide on the vascular wall.[8] Mercuric chloride toxicity has been found to be prevented with vasodilator compounds, oxygen radical scavengers, and nonsteroidal anti-inflammatory agents, leading to the conclusion that mercuric chloride-induced vasoconstriction may be central to its nephrotoxicity and may be mediated by the formation of superoxide anions, stimulating the production of a cyclooxygenase-derived vasoconstrictor agent, reducing endothelial vasodilator activity.[9] Myoglobin selectively reduces blood flow to the outer medulla.[10] The maintenance of normal renal perfusion to the medulla is very dependent on prostaglandin synthesis. Nonsteroidal anti-inflammatory agents and radiocontrast agents reduce outer medullary blood flow.[11,12] Medullary oxygenation in rats is reduced by inhibition of the synthesis of prostaglandins and nitric oxide, as well as by intravenous injection of radiocontrast agents.[13] It has been shown that survival of renal medullary interstitial cells is dependent on prostaglandin synthesis, with an increase in apoptosis of these cells with water deprivation when cyclooxygenase-2–specific inhibitors are used. Thus, nonsteroidal anti-inflammatory agents that block cyclooxygenase-2 could contribute to papillary injury.[14] Chronic administration of nonsteroidal anti-inflammatory agents to animals leads to papillary necrosis and papillary necrosis has been associated with chronic analgesic abuse in patients.[15]

Endothelial injury, together with leukocyte activation, both characteristic of ischemic injury and sepsis, leads to enhanced leukocyte-endothelial interactions that can lead to the generation of local inflammation and secondary effects of inflammatory mediators as well as the physical blockade of local blood flow resulting in secondary ischemia. Nephrotoxic nephritis is an example of how this can occur at the level of the glomerulus. In this model chemokines, produced locally by the mesangial cell and glomerular epithelial cell, have been implicated in monocyte recruitment.[16] At the level of the endothelial cell itself perhaps the best-studied toxin is Shiga toxin. Shiga toxin–producing *Escherichia coli* is a causative agent of the epidemic form of hemolytic uremic syndrome, the most common cause of acute renal failure in children. This syndrome is characterized by microangiopathic lesions as a result of endothelial injury and leukocyte activation. Shiga toxin-2 increases the expression of the chemokines, interleukin-8 and monocyte chemoattractant protein-1 (MCP-1), which have been shown to play an important role in the adherence of leukocytes and transmigration of leukocytes across the endothelium.[17]

LEUKOCYTE INFILTRATION AND ADHESION MOLECULES

Nephrotoxic or ischemia-reperfusion injury result in an inflammatory response, which results in leukocyte infiltration, tissue edema, and compromise of microvascular blood flow. There are many mechanisms by which leukocytes potentiate renal injury, including the generation of reactive oxygen species and the synthesis of phospholipase metabolites, which are important modulators of vascular tone. Enhanced leukocyte-endothelial interactions can lead to outer medullary ischemia and also may lead to interstitial fibrosis accounting for long-term sequelae of nephrotoxicant exposure. The infiltration of leukocytes into tissues is dependent on the enhanced expression of adhesion molecules, including selectins and integrins.[18] Integrins interact with immunoglobulin-like adhesion molecules such as interstitial cell adhesion molecules or vascular cell adhesion molecules. We have reported that intercellular adhesion molecule-1 (CD54) messenger RNA expression is markedly up-regulated after exposure of rats to cisplatin. Pretreatment of animals with anti-CD54 antibody resulted in improvement in renal function, mortality, and

histologic abnormalities in animals exposed to cisplatin.[19] The antibody also reduced the amount of tissue myeloperoxidase that was present after cisplatin treatment. Myeloperoxidase is the marker for tissue leukocytes.

Chemokines, which recruit and activate leukocytes and selectins, are up-regulated by inflammatory cytokines, such as interleukin-1β and tumor necrosis factor (TNFα). There are marked increases in serum, urine, and kidney levels of TNFα after cisplatin exposure. This is associated with increases in tissue messenger RNA levels of TGF-α, RANTES, MIP-2, MCP-1, TCA3, interleukin-1β, and intercellular adhesion molecule-1 in kidneys from cisplatin-treated animals.[20] These increases in cytokine and adhesion molecule messenger RNAs as well as the renal functional consequences of cisplatin were reduced by pretreatment with agents that inhibited TNFα production. Thus, the authors[20] concluded that TNFα plays a central role in the activation of this cytokine response and also in the pathogenesis of cisplatin renal injury, which is mediated at least in part by proinflammatory cytokines and chemokines. Local TNFα synthesis also has been implicated as an early and pivotal event in renal ischemic-reperfusion injury.[21]

TUBULE EPITHELIAL CELL INJURY

Much of the experimental work on nephrotoxicity has centered on the proximal tubule. There are features of this nephron segment that may make it particularly vulnerable to toxicity. Both the S3 segment and the medullary thick ascending limb (MTAL) are located in a region of the kidney that is marginally oxygenated under normal conditions and to which blood flow is reduced in the setting of some nephrotoxicants and in the postischemic period. Although both the S3 segment and MTAL metabolically are quite active with high transport activity, the metabolic characteristics of the 2 segments differ substantially. Proximal tubules have lower glycolytic capacities than distal tubules under conditions of oxygen deprivation.[22,23] MTAL lactate production increases by 1,400% in response to antimycin, which blocks mitochondrial respiration, whereas isolated rat proximal tubules do not produce lactate either under control conditions or in the presence of antimycin.[24] Consistent with the superior glycolytic capacity of the MTAL, glycolytic enzymes are more abundant in distal nephron segments. Thus, the MTAL has a greater capacity to generate adenosine triphosphate via an up-regulation of glycolysis when oxidative metabolism is impaired owing to, for example, mitochondrial toxicity associated with a nephrotoxicant.[25] There are other features of the nephron segments that may make them particularly susceptible to a certain nephrotoxicant. Mercuric chloride, for example, forms a conjugate in the liver with glutathione, which then accumulates in the kidney causing toxicity, particularly to the S3 segment of the proximal tubule. This segment has the highest γ-glutamyl transpeptidase and cysteine transport activity in the kidney.[26]

SUBLETHAL INJURY TO THE TUBULE

Although proximal tubule cell death is a major component of toxicity related to many nephrotoxicants, proximal tubular dysfunction without cellular necrosis or apoptosis is also an important aspect of the cellular response to nephrotoxins. Significant pathophysiologic consequences, such as changes in directional solute reabsorption and paracellular permeability, can occur even if the tubule cell does not succumb to the toxic insult. Sublethal injury to renal epithelial cells in vitro, associated with adenosine triphosphate depletion, results in loss of functional integrity of the tight junction, loss of cell polarity, and impairment of the epithelial permeability barrier.[27] In the case of cadmium toxicity, for example, there may be very subtle morphologic changes of injury. Yet there is very significant proximal tubule dysfunction with increased excretion of β_2-microglobulin in the urine, accompanied by aminoaciduria, glycosuria, and renal tubular acidosis.[28] Carboplatin, which was developed to have less renal toxicity than cisplatin, interferes with the kidney's ability to absorb magnesium. Agents such as amphotericin can markedly affect the function of the distal nephron resulting in renal tubular acidosis, hypomagnesemia, and hypokalemia.[29] Lithium and democylocyline can affect the vasopressin-responsiveness of the collecting duct and interfere with the kidney's ability to concentrate the urine, leading to a state of nephrogenic diabetes insipidus. Lithium down-regulates the vasopressin-regulated water channel aquaporin-2, which normally is expressed in the apical membrane of the collecting duct.[30]

MITOGEN-ACTIVATED PROTEIN KINASES

The mitogen-activated protein (MAP) kinases are an important family of cellular proteins that are modulated in activity in response to ischemia and toxins and cellular stress in general. Furthermore, there is evidence that these proteins may play an important role in the determination of cellular fate in the setting of stress in the kidney as well as other organs.[31,32] All eukaryotic cells have multiple MAP kinase pathways. There are 3 major MAP kinase cascades that have been characterized in mammalian cells to be activated by growth factors, environmental stress, and inflammatory cytokines.[33] These kinase pathways as well as other cellular signaling pathways are critically important for the regulation of transcriptional events.[34]

A toxic insult to the kidney is characterized by a number of responses that activate cellular MAP kinase systems. These include the generation of growth factors,[35] inflammatory cytokines, and a stereotypic cellular response in the face of environmental stress. The best characterized of these MAP kinase pathways is the extracellular signal-regulated kinase (ERK) cascade. Numerous studies have shown that the ERK cascade is critical to the mitogenic response, to cellular differentiation, and, in some cells, to the induction of hypertrophy. Cephaloridine increases ERK activation in renal cortical slices[36] and aminoglycosides do the same in proximal tubule cells.[37] The ERKs are activated by growth factors and many other agonists, including vasoactive peptides, but also can be activated by nephrotoxins. Aminoglycosides can up-regulate ERK in kidney epithelial cells.[37] On activation, ERK proteins translocate to the nucleus.[38] ERKs phosphorylate and activate several members of the Ets-domain transcription factor family, including Elk-1, SAP1, Ets-1, and Ets-2.[38] Elk-1 and the related SAP-1 and SAP-2 are ternary complex factors, which form a complex with serum response factor and together bind to the promoter of a number of genes, including c-*fos*, that contain the serum response element.[39] The nuclear localization of ERK, in addition to a role in transcriptional regulation, also may serve as a cellular mechanism to sequester ERK from MEK1, its cytoplasmic activating protein. The nucleus is also a site for inactivation of ERK by phosphatases, including MKP1 and MKP2, both of which are induced by activation of the ERK pathway, are localized to the nucleus, and possess ERK docking sites.[40]

The 2 other MAP kinase cascades are activated by cellular stress associated with nephrotoxins[41] or ischemia/reperfusion[42] and have been implicated in the apoptosis that is associated with exposure of epithelial cells to nephrotoxins. These kinases are activated only minimally by growth factors but activated markedly by genotoxic stress, osmolar stress, mechanical stretch, shear stress, and inflammatory cytokines (TNFα and interleukin-1β). One of these 2 families of kinases, which has 54- and 48-kd isoforms encoded by at least 3 genes, has been designated either stress-activated protein kinases (SAPKs), because they are activated by cellular stress, or c-Jun N-terminal kinases (JNKs), based on the ability of the kinases to phosphorylate the amino terminus of c-Jun, with the latter term more frequently used to refer to these kinases. The activity of JNKs/SAPKs are increased markedly with ischemia/reperfusion.[42] The other stress kinase family includes p38α, the mammalian homolog of HOG-1, a yeast kinase involved in the response to osmolar stress, and 3 related kinases, p38β, p38δ, and p38δ. Similar to ERK1/2, the SAPKs and p38 are proline-directed and require phosphorylation on both tyrosine and threonine residues for activation.[33] We found that the SAPKs are not activated by ischemia alone, but are activated markedly by reperfusion of the ischemic kidney.[42] The SAPKs phosphorylate and activate a bZIP transcription factor, ATF-2. The SAPKs are the predominant ATF-2 transactivation domain kinases in the postischemic kidney, suggesting that the JNK/SAPKs are physiologically relevant ATF-2 kinases.[43] ATF-2 can form homodimers, or heterodimers with other members of its family, ATF-3 and CREB, or with c-Jun or nuclear factor-κB, suggesting it may play a role in the activation of transcription from many promoters. The role of JNK activation in nephrotoxicity is not well studied. As described previously, we reported that there is marked up-regulation of this kinase with ischemia. Little is known, however, about the regulation of this family of MAP kinases with toxins. In the inner ear, where gentamicin toxicity is problematic, as it is in the kidney tubule cell, ototoxicity leads to JNK activation and apoptosis in the hair cells.[44] Systemic administration of CEP-1347, a JNK inhibitor, attenuates gentamicin-induced decrease of auditory sensitivity, cochlear hair cell

damage, and reduces the extent of hair cell loss in the ampullary cristae after gentamicin administration. In this study,[40] the authors implicated the JNK cascade as an important mediator of cellular toxicity.

MEKK1, the upstream activator of JNK, is a 196-kd protein that, when cleaved by caspase-3–like proteases, generates an active COOH-terminal kinase domain. Induced expression of activated MEKK1 kinase domain in HEK293 human kidney cells induces apoptosis and potentiates cisplatin-induced and TNFα-induced cell death of L929 fibrosarcoma cells.[45]

p38s are activated by many of the same stimuli that activate the JNK/SAPK pathway, yet the pathways are regulated independently. Some stresses activate the p38 pathway in preference to the JNK/SAPK pathway and other stresses preferentially activate the JNK/SAPK pathway. The p38s are key regulators of inflammatory cytokine production. It recently has been reported that a scaffolding protein, TAB1, can activate p38α. This is particularly interesting because TAB1 does not have any known catalytic activity and hence is not a MAP kinase kinase.[46] This provides further evidence that scaffolding proteins play critical roles, not only in localization of the MAP kinases within the cell[40] but also in regulating their activation.

An important way in which the JNKs and p38 can contribute to nephrotoxin injury is through their effects to stabilize and enhance translation of mRNAs encoding proinflammatory proteins.[33] As described earlier, cisplatin nephrotoxicity is associated with increased inflammation.[19] Cadmium exposure in humans and experimental animals results in degeneration and necrosis of tubular epithelial cells followed by interstitial inflammation and eventual regeneration of proximal tubular cells.[47] A large number of other nephrotoxins result in interstitial inflammation.

Di Mari et al[32] have argued that ERK is activated in distal tubule cells but not in the proximal tubule cells after ischemia/reperfusion. P54 JNK is activated in both. If, as has been proposed, ERK expression is protective, then this imbalance in ERK and p54 SAPK activity may account partly for the differential susceptibility of the proximal and distal tubules to injury. Di Mari et al[32] found that, whereas JNK is activated in both the cortex and inner stripe of the outer medulla, the ERK pathway is activated only in the inner stripe in which the thick ascending limbs predominate. Together with their in vitro data that showed that proximal cells were more susceptible to oxidant injury than thick ascending limb cells and ERK activation only occurred in thick ascending limb cells, the investigators proposed that cell survival in the postischemic kidney relied on ERK activation. We have found recently, by using immunocytochemical techniques, that ERK activation, as measured with phospho-specific antibodies, is upregulated in the thick ascending limb after ischemia/reperfusion,[48] consistent with the hypothesis put forth by Safirstein et al. Safirstein, DiMari, and colleagues found that administration of N-acetyl cysteine inhibited the ischemia-induced increase in SAPK/JNK activity and partially protected the kidney against ischemic injury.[49] Whether the protection was related to the decrease in SAPK/JNK activity or to other anti-oxidant effects of N-acetyl cysteine was not established. In studies exploring mechanisms of ischemic preconditioning in the kidney we have found that increased ERK activation relative to JNK activation (Fig 1) is associated with functional protection of the kidney and decreased postischemic leukocyte infiltration.[50] In these studies prior exposure to ischemia protects the kidney against a subsequent second ischemic exposure. We also see protection when the first stress is transient ureteral obstruction.[51]

ENDOPLASMIC RETICULUM STRESS RESPONSE

An important component of the stress response of the cell resides in the endoplasmic reticulum (ER). It is in the ER that almost all soluble and membrane proteins of the cell are processed. Proteins enter the ER in an unfolded state as linear polypeptides. In the ER there are resident enzymes and chaperones. The ER is also the site of degradation of unfolded proteins. Therefore, a polypeptide that enters the ER either can be processed or degraded. A number of conditions will increase the amount of unfolded protein in the ER. For example, if glycosylation is inhibited by tunicamycin, there will be increased amounts of these proteins. If the redox state of the ER lumen is altered by reducing agents such as dithiothreitol or luminal calcium concentration increases, there will be increased amounts of unfolded protein in the lumen. In any of these cases in which an increased burden of unfolded proteins exceeds the ER's ability to

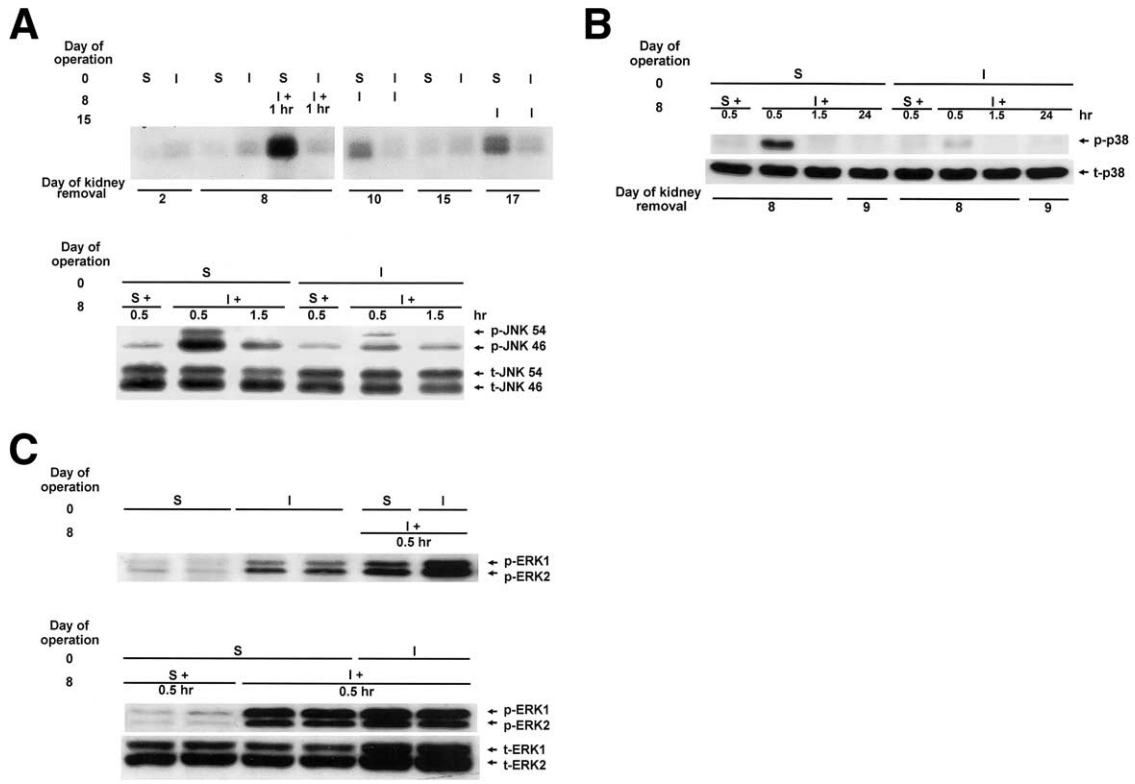

Fig 1. JNK kinase activity and phosphorylation of JNK, p38, and ERK1/2 in mouse kidneys exposed to ischemia (I) or sham surgery (S) on day 0, followed in some cases by ischemia on day 8. (A) Upper panel: JNK activity was assayed in kidneys removed on 2, 8, or 15 days, or 1 hour after an ischemic period on day 8. In addition, JNK activity was measured in kidneys removed on day 10, 2 days after ischemia on day 8. JNK activity also was measured on day 17 in kidneys exposed to ischemia or sham surgery on day 0 and ischemia on day 15. Lower panel: Western blot analysis of phospho-JNK and total JNK in kidneys exposed to sham or ischemic surgery on day 0, followed on day 8 by sham surgery or ischemia. Kidneys then were removed at either 0.5 or 1.5 hours after the procedure on day 8. (B) Western blot analysis of phospho-p 38 and total p 38 in kidneys exposed to sham or ischemic surgery on day 0, followed on day 8 by sham surgery or ischemia. Kidneys then were removed at either 0.5, 1.5, or 24 hours after the procedure on day 8. (C) Western blot analysis of phospho-ERK1 and 2 and total ERK 1 and 2 in kidneys exposed to sham surgery or ischemia on day 0, followed on day 8 by sham surgery or ischemia. Kidneys then were removed at 0.5 hours after the procedure on day 8. There is more ERK phosphorylation and less JNK and p38 phosphorylation in ischemia-preconditioned kidneys in which the functional injury was much less after ischemia. Reprinted with permission from Park et al.[50] © 2001 The American Society for Biochemistry & Molecular Biology.

deal with them results in a state of ER stress. Proteins such as grp78 or the ER-specific caspase 12 have been used as markers of ER stress. Recently, it was reported that cisplatin has non-DNA–mediated mechanisms to induce apoptosis that are characterized by an increase in ER stress.[52]

We have found that the ER stress response confers protection against oxidant injury to the cell,[54] following on the extensive work of Stevens and colleagues.[53] We investigated the role of the ER stress response in intracellular Ca^{2+} regulation, MAP kinase activation, and cytoprotection in LLC-PK_1 renal epithelial cells in an attempt to identify the mechanisms of protection afforded by ER stress.[54] Cells pretreated with DTTox, tunicamycin, thapsigargin (which inhibits the ER Ca^{2+}-adenosine triphosphatase) or the Ca^{2+} ionophore, A23187, express ER stress proteins and are resistant to subsequent H_2O_2-induced cell injury. In addition, ER stress preconditioning prevents the increase in intracellular Ca^{2+} concentration that normally follows H_2O_2 exposure. Stable overexpression of the ER calcium binding protein calreticulin in LLC-PK_1 cells, pkCRT cells, prevents an increase in intracellular free calcium concentration ($[Ca^{2+}]_i$) normally observed with H_2O_2, and pkCRT cells also were more resistant to H_2O_2-induced cell injury relative to pkNEO cells. This

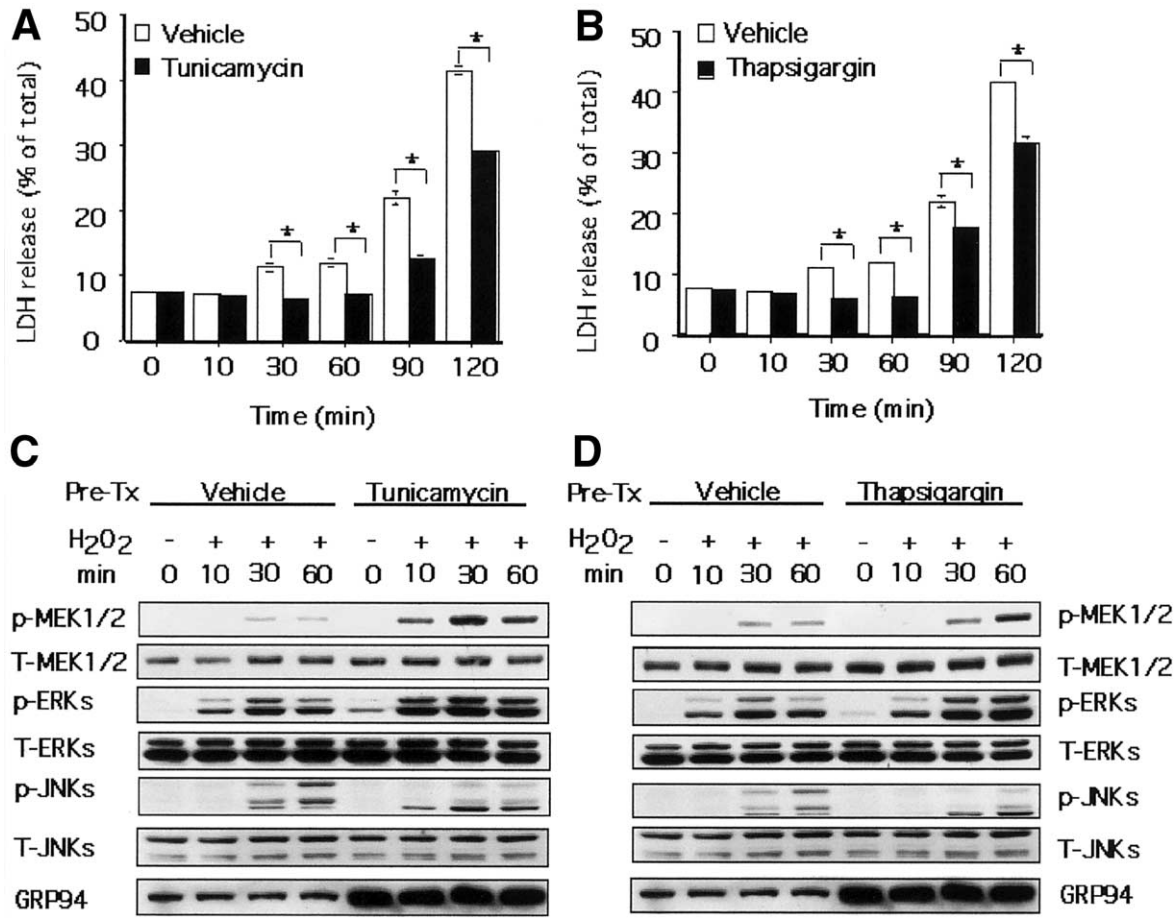

Fig 2. ER stress caused by tunicamycin or thapsigargin modulates MAPK signaling responses to oxidative stress. (A) Nonpretreated (vehicle Pre-Tx) LLC-PK$_1$ cells or cells preconditioned with tunicamycin (1.5 μg/mL, 16 h) or (B) thapsigargin (0.3 μg/mL, 16 h) to induce ER stress were washed and returned to Dulbecco's modified Eagle's medium plus 0.1% fetal calf serum for 3 hours before directly adding 250 μmol/L H_2O_2. Cell injury at different times after addition of H_2O_2 was determined by measuring lactate dehydrogenase release. The data are the means ± SE of data from duplicate measurements from 3 different experiments (n = 3). Significant differences (*P < .05) between 2 groups at various time points were determined by Student's t test. (C and D) In parallel, cells were lysed at different times before significant cell injury. MEK1/2, ERK, and JNK activation in cell lysates were determined with phospho-specific antibodies that recognize phosphorylated MEK1/2, ERK, or JNK. Total MEK1/2, ERK, and JNK also were measured by specific antibodies. The expression of GRP94 confirms the induction of ER stress response by tunicamycin and thapsigargin. The blot shown is representative of 3 separate experiments in which similar results were observed. Reprinted with permission from Hung et al.[54] © 2003 The American Society for Biochemistry and Molecular Biology.

protection was associated with maintenance of low $[Ca^{2+}]_i$ after H_2O_2 treatment. Without H_2O_2 treatment, there was no difference in $[Ca^{2+}]_i$ in pkCRT and pkNEO cells. However, 2 hours after H_2O_2 treatment there is a significant increase in $[Ca^{2+}]_i$ in pkNEO cells but not in pkCRT cells. Therefore, similar to GRP78, an ER chaperone protein induced by ER stress, calreticulin attenuates cell injury and prevents the increase of $[Ca^{2+}]_i$ normally observed after H_2O_2 treatment. Stable transfection of cells with antisense RNA targeted against GRP78 (pkASgrp78 cells) prevents GRP78 induction, disables the ER stress response, and sensitizes cells to H_2O_2-induced injury. Stable expression of pkASgrp78 also prevents the development of tolerance to H_2O_2 that normally occurs with preconditioning. ERK and JNK are transiently (30–60 min) phosphorylated in response to H_2O_2. ER stress preconditioned cells have more ERK and less JNK phosphorylation than control cells in re-

sponse to H_2O_2 exposure (Fig 2). Pre-incubation with a specific inhibitor of JNK activation or adenoviral infection with a construct that encodes constitutively active MEK1, also protects cells against H_2O_2 toxicity. MEK1-DD, a constitutively active mutant of MAPK/ERK kinase 1 (MEK1), the upstream activator of ERK1/2, has serine 218 and 222 replaced by aspartic acid residues as previously described.[55] A recombinant adenoviral vector, AdMEK1-DD carrying the MEK1-DD complementary DNA, was used to express MEK1-DD in LLC-PK1 cells. In contrast, the pkASgrp78 cells have less ERK and more JNK phosphorylation with H_2O_2 exposure. Expression of constitutively active ERK also confers protection on native as well as pkASgrp78 cells. These results indicate that GRP78 plays an important role in the ER stress response and cytoprotection.

To evaluate if ERK activation can rescue the pkASgrp78 cells exposed to H_2O_2, we infected the pkASgrp78 cells with AdMEK1-DD. After adenoviral infection, the pkASgrp78 cells with constitutively activated ERK are more resistant to oxidative injury than are cells infected with adenovirus carrying *LacZ* gene only. Thus, the enhanced sensitivity of pkASgrp78 cells to oxidative stress is associated with less phosphorylation of ERK in response to H_2O_2, an effect that can be overcome by expression of constitutively active MEK1. Therefore, ERK pathway activation is a downstream effector of the protective response mediated by ER stress and GRP78 expression. The ER stress response modulates the balance between ERK and JNK signaling pathways to prevent cell death after oxidative injury.

Pre-incubation of LLC-PK_1 cells with a specific phosphatidylinositol-3 kinase inhibitor LY294002 (10 μmol/L) completely abolished the phosphorylation of Akt/PKB but did not alter the cell injury seen after H_2O_2 exposure. Thus, phosphorylation of another cellular kinase that has been associated with determination of cell fate, Akt/PKB, does not influence cell survival in LLC-PK_1 cells treated with H_2O_2.

CONCLUSION

Renal injury associated with nephrotoxins involves diverse processes having effects on the distribution of renal blood flow as well as various effects on the tubule cell. Each toxin has distinct mechanisms responsible for injury but there are features common to many toxins that include to varying extents: local ischemia, endothelial injury, inflammation, activation of cell MAP kinase pathways, and activation of the cellular stress response, which provide hope that there may be common therapies of protection. Our understanding of the cellular and molecular aspects of nephrotoxicity lags slightly behind our understanding of the mechanisms of ischemic injury although in neither case are the mechanisms in humans understood to the extent that we have been able to establish effective preventive and therapeutic pharmacologic interventions.

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