Recent Advances in the Pathophysiology of Ischemic Acute Renal Failure

JOSEPH V. BONVENTRE* and JOEL M. WEINBERG†

*Renal Division, Brigham and Women’s Hospital, and Department of Medicine, Harvard Medical School, and the Harvard–Massachusetts Institute of Technology, Division of Health Sciences and Technology, Charlestown, Massachusetts; and †Division of Nephrology, Department of Internal Medicine, University of Michigan and VA Medical Center, Ann Arbor, Michigan.

As covered in the preceding sections, acute renal failure (ARF) is a syndrome associated with high mortality in humans. Current therapy is limited to supportive measures and preventive strategies, none of which have been definitively shown to alter mortality. Ischemic ARF is often associated with multiple organ failure and sepsis. Despite the complexity of multi-organ illness, the presence of ARF independently carries a marked increase in mortality (1). This review is a brief treatment of our current understanding of the pathophysiology of experimental ischemic ARF and some recent advances in this field. We will first discuss the vasculature. In this context we will consider vasoconstriction as well as endothelial injury. This will lead naturally into the important contribution of inflammation. The response of the epithelial cell and its role in the pathophysiology will then be discussed. In this context, the effects of ischemia on survival of these cells will be supplemented by a discussion of their role as potential contributors to the injury itself. Since ARF is a reversible disease, the manner in which the epithelium is regenerated both anatomically and functionally will be described. We will then discuss the property of the kidney to undergo preconditioning whereby it is protected against a subsequent ischemic insult. Finally we will discuss the status of biomarker research, as the latter is critical to allow for early diagnosis of ARF and evaluation of therapeutic effectiveness as well as for the rational design of human clinical trials.

Vascular Reactivity and Endothelial Injury in the Post-ischemic Kidney

In both humans and experimental animals (Figure 1), reductions in GFR have been attributed to persistent vasoconstriction, potentially contributed to by activation of tubuloglomerular feedback as a result of enhanced delivery of solute to the macula densa. In fact, ischemic ARF has been referred to as “vasomotor nephropathy” in the past (2). It is known that there is increased basal tone, increased reactivity to vasoconstrictive agents, and decreased vasodilatory responses in arterioles taken from post-ischemic kidneys when compared with those taken from normal kidneys (3). Some have argued that persistent preglomerular vasoconstriction is at the heart of impaired GFR (2). Increased solute delivery to the distal nephron can be explained in part by loss of cell polarity in the proximal tubule with mislocalization of the Na⁺K⁺-ATPase and impaired tight junction integrity resulting in decreased apical to basolateral transcellular sodium reabsorption (4,5). A reduction in total renal blood flow evenly distributed to the tubules is likely not to be the explanation for the pattern of damage to tubular cells, especially given the low oxygen extraction necessary to support tubule metabolism in the cortical proximal tubules. It is reduction in regional blood flow to the outer medulla that is most important. It has been known for some time that there is less flow to the medullary than the cortical capillaries in the post-ischemic kidney (6). In addition, endothelial injury results in cell swelling and enhancement of expression of cell adhesion molecules. This, together with leukocyte activation, leads to enhanced leukocyte–endothelial interactions, which can promote injury and swelling of the endothelial cell, physically impede blood flow, contribute to the production of local factors promoting vasoconstriction, and add to the effects of vasoconstriction on local blood flow and tubule cell metabolism (7,8). Disorganization of the actin cytoskeleton has been found to be present in the arteries, arterioles, and mural cells or pericytes of vasa recta of the kidney after an ischemic insult (9). It has been proposed that this change in the cytoskeleton may play an important role in the loss of autoregulation of renal blood flow and abnormal vascular reactivity, which is a characteristic of post-ischemic ARF (9). There are areas where the endothelial cells are lost. Goligorsky and colleagues (10) have recently reported that infusion of human umbilical vein endothelial cells after release of the renal artery clamp protected the kidney against subsequent loss of function via production by the endothelial cells of nitric oxide (NO).

Damage to the endothelial layer will result in alterations in the response of arterioles to vasoactive substances. Under normal circumstances, NO generated from the endothelial cell plays an important vasodilatory role. With endothelial damage, the endothelial nitric oxide synthase (eNOS) is inhibited. Conger et al. (11) reported a number of years ago that rather than
a vasodilatory response of the kidney to acetylcholine, the post-ischemic kidney responds with vasoconstriction. In addition to changes in the effectiveness of vasoactive compounds on resistance vessels due to changes in the endothelial cells, alterations in the local levels of vasoconstrictor agents have been implicated in abnormal vascular tone. Vasoconstrictors implicated in the regulation of post-ischemic vascular tone include angiotensin II, thromboxane A2, leukotrienes, sympathetic nerve activity, nitric oxide, PGE2, acetylcholine, and bradykinin. Endothelial and vascular smooth muscle cell structural damage and Leukocyte-Endothelial adhesion vascular obstruction, leukocyte activation, and inflammation.

**Pathophysiology of Ischemic Acute Renal Failure**

**MICROVASCULAR**

- Vasoconstriction *in response to:* endothelin, adenosine, angiotensin II, thromboxane A2, leukotrienes, sympathetic nerve activity
- Vasodilation *in response to:* nitric oxide, PGE2, acetylcholine, bradykinin
- Endothelial and vascular smooth muscle cell structural damage
- Leukocyte-Endothelial adhesion vascular obstruction, leukocyte activation, and inflammation

**TUBULAR**

- Cytoskeletal breakdown
- Loss of polarity
- Apoptosis and Necrosis
- Desquamation of viable and necrotic cells
- Tubular obstruction
- Backleak

**Figure 1.** Interacting microvascular and tubular events contributing to the pathophysiology of ischemic acute renal failure (ARF).
fusion after a period of ischemia in the rat. There was also a partial transient compromise of the patency of the peritubular capillaries upon reperfusion, with rapid recovery. Basile and colleagues (22) have found reductions in the number of microvessels in the inner stripe of the outer medulla at 4, 8, and 40 wk after a prolonged period (60 min) of ischemia in the rat. This is associated with tubulointerstitial fibrosis and altered concentrating ability.

Activation of the endothelial cell with upregulation of adhesion molecules, as well as injury to endothelial cells leading to cell swelling and loss of the patency of the endothelial barrier, will potentiate interactions with leukocytes and platelets and mechanical obstruction to the small vessels. The leukocytes are activated by a number of local factors, including cytokines, chemokines, eicosanoids, and reactive oxygen species (ROS), which result in upregulation of adhesion molecules, which are counter-receptors to the adhesion molecules expressed on the activated endothelium. Furthermore, exposure of leukocytes to cytokines either locally or systemically can reduce their deformability and enhance the tendency for them to be sequestered (23). Sequestered leukocytes can then enhance the injury via a positive feedback pathway in which they generate ROS and eicosanoids, which can further the inflammatory responses and enhance vascular tone. Functional and morphologic protection of the kidney with agents designed to prevent leukocyte-endothelial adhesive interactions or in animals that do not produce ICAM-1 (24), an important endothelial adhesion molecule, is associated with reductions in leukocyte sequestration.

Some controversy exists as to whether neutrophils or mononuclear cell infiltration are more important in ischemia/reperfusion injury (25–28). Tissue injury is ameliorated by prevention of neutrophil accumulation in some studies, but not all (24,29–31). In addition, however, neutrophil deletion models may not adequately differentiate neutrophils from macrophages and T lymphocytes (25). Myeloperoxidase activity, which is elevated soon after the ischemic insult, may originate from macrophages as well as neutrophils (32). The infiltration of macrophages and T lymphocytes may predominate over neutrophils in the later recovery phase of ARF (25,27,33). There are also likely to be species differences, however. In our experience, neutrophils are more readily seen post-ischemia in the mouse than they are in the rat, for example. Blockade of T cell CD28-B7 co-stimulation with CTLA.4Ig resulted in protection against ischemic injury in rats and significant inhibition of T cell and macrophage infiltration and activation in situ (34). After ischemia, there is an upregulation of B7–1 protein expression on the ascending vasa recta (33). T cells and monocytes/macrophages were found trapped in the vasa recta as early as 2 h after reperfusion. Accumulation of these cells was reduced by pretreatment with anti-B7–1 but not anti-B7–2 antibodies (33). Consistent with a causal role for T lymphocytes, CD4+/CD8+ knockout mice were protected from ischemia/reperfusion injury (26). However, (RAG)-1–deficient mice, which have no T or B cells and do not produce immunoglobulins or T cell receptor proteins, were not protected (28).

Activation of the coagulation pathways is likely to contribute to compromise of the medullary vasculature and hence functional post-ischemic injury. We have reported that an oral inhibitor of platelet activating factor is protective against ischemic injury in the rat (35). Endothelin-1 also modulates neutrophil adhesion after coronary artery bypass graft, and enhances leukocyte rolling and adhesion in rat mesenteric vessels, an effect abrogated by anti-P-selectin antibody (36). A similar effect in the microvessels of the kidney, especially in the vessels of the outer medulla, might enhance leukocyte–endothelial adhesion compromising blood flow to particularly vulnerable tissue regions.

Chemokines, which are chemotactic and immunomodulatory for leukocytes, are upregulated by inflammatory cytokines, such as IL-1 and TNF-α (37). Renal TNF-α mRNA is increased after only 30 min of ischemia, and the TNF-α transcription factor, NF-κB, is activated after 15 min of ischemia (38). Infusion of a TNF-α–binding protein decreases TNF-α bioactivity and neutrophil infiltration and preserves renal function, suggesting that local TNF-α synthesis may be an early and pivotal event in renal ischemia/reperfusion injury (38). ROS can upregulate chemokine expression. Transgenic mice that overproduce the antioxidants, intracellular, and extracellular glutathione peroxidases, have less induction of the chemokines KC and macrophage inflammatory protein-2 (MIP-2), less neutrophil infiltration, and less functional injury after ischemia (39). Agents whose effects have been usually attributed to vasoconstriction, such as angiotensin II, may actually act primarily by upregulating the production of chemokines by endothelial cells (40), thus enhancing leukocyte-endothelial interactions. NO inhibits TNF-α–induced adhesion of neutrophils to endothelial cells, which represents another mechanism through which NO can be protective (41).

Complement may also play a role in potentiation of leukocyte-endothelial interactions. In a number of organs exposed to ischemia/reperfusion, complement-dependent endothelial upregulation of adhesion molecules with resultant neutrophil accumulation in the microvasculature has been implicated as a proximal effector mechanism for complement-mediated injury (42). Others, however, have argued that the primary effect of complement in kidney ischemia/reperfusion injury is on the epithelial cell due to a direct effect of the membrane attack complex of complement (43). Inhibition of C5 results in protection against renal dysfunction and late inflammation, as measured by neutrophil influx, induction of the murine CXC chemokines MIP-2, KC (IL-8), and lipopolysaccharide-induced CXC chemokines, and reduction of late apoptosis. By contrast, early apoptosis is not affected (44).

The Tubule Cell as a Contributor to the Inflammatory Response

The renal epithelial cell can produce fractokine (CX3CL1), a transmembrane protein with a CX3 chemokine motif attached to a mucin stalk (45). Fractokine is known to induce adhesion and migration of leukocytes. This could facilitate monocyte-induced cell injury. Renal tubular cells can also produce a number of proinflammatory cytokines, including TNF-α, interleukin-6 (IL-6), TGF-β, and chemotactic cytokines (chemo-
Injury in the Kidney

Spatial and Temporal Patterns of Parenchymal Cell Injury in the Kidney

As discussed recently by Sutton et al. (54), the development and progression of parenchymal cell injury in the kidney during ischemic acute renal failure can be viewed as a continuum of events. During the initiating period of ischemia, the most global effects of ATP depletion and failure of perfusion occur. During a subsequent extension phase, post-ischemic reperfusion allows for both recovery and additional injury events that are ATP- and perfusion-dependent in reperfused areas. At the same time, however, the inflammatory processes and primary ischemic injury to vascular cells discussed in the preceding sections of this review result in localized but potentially lengthy prolongation of ischemia that substantially increases the severity of injury in the areas affected. In the commonly studied vascular clamp small animal models of ischemia, the outer medulla, where the S3 segment of the proximal tubule and the medullary thick ascending limb (MTAL) are located, is most affected by this compromised reflow (55). The sensitivity of the outer medulla derives from a combination of its microvascular architecture and the oxygen demands of the tubule segments located there (6,56). During the following maintenance phase, large areas of severe local ischemia may no longer be present, but cell injury continues as a result of surrounding inflammation and the effects of the prior insults on intrinsic cellular responses such as apoptosis. The efficacy of the diverse array of antiinflammatory maneuvers and other approaches that act primarily at the level of the microvasculature to ameliorate ARF in these models, when applied during the extension phase in the first 6 to 12 h after the initiating insult, indicates that injury occurring at this time is decisive for the ultimate outcome. Whether a similar balance between events during “initiation” and “extension” with regional localization of injury due primarily to inflammation and prolonged impairment of outer medullary perfusion can account for human ischemic acute renal failure, continues to be debated (55,57).

The cellular alterations that are most evident and amenable to study occur in tubule cells, particularly the proximal tubule. However, parallel processes derived from the same metabolic events occurring in the vasculature in endothelial and smooth muscle cells are potentially even more critical to the outcome. Effects on the endothelium and smooth muscle determine the duration and distribution in the kidney of the further injury that occurs during the “extension” phase and contribute to the decrease of GFR subsequently during the “maintenance” phase (54).

Cell Death

Necrosis. The severe ATP depletion that occurs during the initiation phase and in the most poorly reperfused areas during extension disrupts cellular Na+, K+, volume, and Ca2+ homoeostasis (58), causes accumulation of phospholipid metabolites (58), and produces generalized protein dephosphorylation (59) and widespread protein redistribution (4) and aggregation (60). Cell death by necrosis, as seen most prominently in the proximal tubule, is usually considered the nonspecific outcome of the chaotic, confluence of these events. However, necrotic cell death can occur as the result of ATP depletion-induced opening of a plasma membrane “death channel” well before cell injury is particularly advanced or irreversible in other respects (61). This channel is normally kept closed in ischemic tissue by the presence of high levels of tissue glycine (62) and decreased pH (58). During extension, in areas that remain severely underperfused, necrosis will occur after periods of greater than several hours despite the presence of glycine and cellular acidosis. In reperfused areas, recovery of pH (63) and washout of glycine allow opening of the death channel in cells that are unable to recover ATP as a result of non-oxidant damage to mitochondrial oxidative phosphorylation mechanisms during ischemia (64) and oxidant damage to mitochondria during reperfusion (65). Oxidant generation and protease activation play major roles in continuing damage.

As comprehensively reviewed in references 66 and 67, there are multiple sources of ROS, including mitochondrial electron transport, cyclooxygenases, lipooxygenases, and mixed-function oxidases of the endoplasmic reticulum, the xanthine oxidase system, and tubule cell plasma membrane NADPH oxidase. ROS react with proteins, lipids, nucleic acids and carbohydrates to contribute to development of necrosis, dysfunction of sublethally injured cells, and generation of the signals for activation of both apoptotic and cell survival pathways. Cellular effects of ROS are amplified during ischemia/reperfusion by the release of catalytically active iron (66,68) and by increased production of NO, possibly by iNOS (69,70), with formation of peroxynitrite from the reaction between NO and superoxide anion. Iron-catalyzed ROS production bypasses the glycine-sensitive death channel to produce necrosis (71). Meprin, a brush border protease selectively expressed in
S3 segments, has a strong, toxic effect and likely acts from both the surface of cells and after the internalization of microvilli (72). Activation of caspases and calpain also contribute to development of necrosis.

It merits emphasis that the differences in reperfusion and oxygenation that determine cellular behavior can occur in microdomains so that adjacent cells are differently affected (73), as well as at the regional level typified by the behavior of the outer medulla (56). Furthermore, as local inflammation worsens, in part driven by inflammatory mediators produced by tubules, well-reperfused areas can become underperfused again.

**Apoptosis.** On pathologic examination of the post-ischemic kidney, necrotic cell death of proximal tubules is initially more prominent than apoptotic cell death for several reasons. One reason is simply that apoptotic cells and their fragments are smaller and more rapidly cleared (74). A second reason is that ATP is required for execution of the full apoptotic pathway (74,75) as well as to delay opening of the glycine-sensitive plasma membrane death channel that results in necrosis (61). ATP that is generated to support the full apoptosis program must be substantially glycolytic in origin because of the compromised mitochondrial ATP production that results from both the ischemic insult and from the mitochondrial de-energization that occurs as apoptosis progresses (76) and glycolytic ATP production is limited in proximal tubules before injury (77). A third and possibly most fundamental reason why apoptosis is not more prominent at the outset is that bax and bak, the two pro-apoptotic proteins that act most directly at the outer mitochondrial membrane in the intrinsic pathway for initiating apoptosis via release of cytochrome c and other proteins from the mitochondrial intermembrane space (76), are downregulated in vivo before injury (78,79). Distal tubule segments, including the MTAL, are more glycolytic than the proximal tubule (77). This contributes to their resistance to necrosis and instead favors development of apoptosis (80,81) or sublethal forms of injury that are associated with production of cytokines, chemokines, and growth factor, which may have paracrine effects on adjacent proximal tubules and/or small vessels (82).

Apoptosis becomes increasingly important over time after the initiating insult. Expression of pro-apoptotic members of the bcl2 family including bax, bak, and bad (78,79,83), as well as caspases (84) increases. There is also increased expression of other components of both the intrinsic (85) and extrinsic pathways (86) for apoptosis. These pro-apoptotic factors are induced in response to a number of consequences of the primary insult, including DNA damage, production of ROS, and generation of ceramide (fully reviewed in reference 87). In general, increases of expression occur in both proximal and distal tubules, although currently available data are limited and in some cases conflicting about preferential localization of specific proteins to different tubule segments (78,88). Surviving but damaged proximal tubule cells can become more “competent” to undergo apoptosis as opposed to necrosis to the extent that increased expression of glycolytic enzymes in dedifferentiated proximal tubule cells (89) provides an ATP source and increases of plasma membrane cholesterol content reduce susceptibility to necrosis (90).

The role of apoptosis and the proteins that mediate it in the development of ARF is being extensively studied. Pan-caspase inhibitors are protective during murine clamp ischemia/reperfusion with a global reduction of necrotic and apoptotic cell death (91). These results suggest a predominant effect of caspases on the inflammatory component of the extension phase. Consistent with this interpretation, inhibition of caspase-1–mediated processing of IL-18 has been implicated (92). A damaging feedback loop intrinsic to tubule cells may also be involved insofar as tubules produced IL-18 and addition of IL-18 to hypoxic isolated tubules accelerates damage (93). It has been suggested that apoptotic tubule cell death may be more predictive of functional changes than necrotic cell death based on observations that functional amelioration of acute renal failure by either guanosine or pifithrin-α, a p53 inhibitor, was associated with decreased apoptosis but no changes in the extent of necrosis (85,94).

DNA damage is a major signal for increased p53 expression and occurs during ischemia/reperfusion as a result of both oxidation and endonuclease-mediated single strand breaks (as opposed to nucleosomal cleavage during apoptosis) (95). Studies using p21 knockout mice have shown that the increases of p21 and resulting cell cycle inhibition that occur during ischemic ARF play an important role in preventing more extensive cell death (96), presumably due to increased and inappropriate cell cycle activity before repair of that damage. Interestingly, the increased cell death in the p21 knockout mice occurs in cortical proximal tubules and is manifested as necrosis (96).

The effects of growth factors including epidermal growth factor, insulin-like growth factor, and hepatocyte growth factor to alleviate ARF, originally considered to act at the tubule cell level by promoting proliferation for repair (97), also involve in large part anti-apoptotic effects on survival pathways (98). An interesting question, which has not yet been addressed, is whether it will be possible to demonstrate selectivity of any growth factor for apoptotic cell death as has been reported for guanosine and pifithrin-α (85,94).

**Sublethal Injury**

Although cell killing is a prominent feature of experimental ischemic acute renal failure, much of the injury relevant to failure of organ function, particularly as expressed in biopsy material available for human acute renal failure (5,57), remains sublethal. During the past decade, there has been substantial progress in understanding some of the mechanisms that figure most prominently in this sublethal injury. Ischemia results in rapid loss of cytoskeletal integrity and cell polarity with mislocalization of adhesion molecules and other membrane proteins such as Na+/K+-ATPase (4). Increases of paracellular permeability lead to backleak of glomerular filtrate (99). The apical actin network is disrupted very early after ischemia. The brush border disappears with shedding and internalization of apical membrane proteins and blebbing of apical membranes (4). Several processes have been implicated in these brush border alterations. Diphosphorylation of ezrin disrupts actin
membrane tethering (59). Actin depolymerizing factor is activated by dephosphorylation and redistributes from the cytosol to damaged microvilli, where it can promote severing and depolymerization (100). Ca$^{2+}$-dependent severing of actin by villin does not occur before lethality (101).

Mislocalization of adhesion molecules (102) leads to detachment of tubule cells from the basement membrane and from each other and contributes, along with the detached microvilli, to the formation of intraluminal aggregations of cells, which together with proteins and glycoproteins such as fibronectin (103), results in tubular obstruction. The behavior of multiprotein complexes at major cell:cell and cell:matrix contact sites has been subject to detailed analysis in several tubule cell systems. Protein dephosphorylation figures importantly in disruption of tight junctions (60) and focal adhesions (104). There is evidence that tyrosine kinase-mediated phosphorylation of β-catenin disrupts adherens junctions (105). Proteins in these complexes as well as others (e.g., Na,K-ATPase) will not all be restored to their proper level of expression and locale during ATP repletion by reuse of the existing proteins and new synthesis at a normal rate together with appropriate targeting is necessary (106). It is likely that there is persistent inappropriately increased protein degradation and turnover in damaged cells. These potentially fruitful areas for study have been only minimally addressed in the context of ischemic injury in the kidney. Increased levels of endoplasmic reticulum chaperones, known to be upregulated in response to accumulation of misfolded or misassembled secretory proteins, were detected in both ischemic kidney and ATP-depleted MDCK cells (107). Golgi spectrin was extensively dissociated in ATP-depleted MDCK cells and clathrin levels and distribution were abnormal and delivery of secretory proteins basolaterally remained impaired at 18 h of ATP recovery (108). The mechanisms for sustained decreases of multiple transport proteins after ischemia/reperfusion (109,110) are poorly understood and potentially derive from a combination of phenotypic alterations in “simplified” recovering tubule cells along with alterations in protein synthesis, targeting, and degradation.

Modification of Injury by Preconditioning

Although long known (reviewed in reference 111), the possibility of conferring protection against subsequent insults by prior injury or preconditioning maneuvers has been an area of increasing interest. This derives from greater appreciation of the efficacy of a variety of approaches, many of which are relatively benign and therefore candidates for practical use, along with the insights that are emerging from gene expression studies on the induced proteins that mediate some of the effects (112). Important potential candidates as mediators of preconditioning, and processes of active investigative interest, include induction of heat shock protein molecular chaperones (113,114) and heme oxygenase (115,116), increases of membrane cholesterol content (90), reduction in the relative activation of JNK as compared with ERK1/2 (117,118), activation of the NOS pathways (118a), stimulation of PPAR receptors (119), induction of endoplasmic reticulum stress proteins (120, 120a), activation of the phosphatidylinositol-3-kinase Akt/ PKB pathway (121), and facilitation of a more open state of mitochondrial K$_{ATP}$ channels (122).

Detailed treatment of the large and growing literature in this field is beyond the scope of this brief review. Several general considerations, however, are pertinent to mention. In addition to direct effects of systemic preconditioning maneuvers on cells in the kidney, circulating factors such as hepatocyte growth factor (123) and IL-10 (124) produced in the liver, can play a major role in modulating both the inflammatory and parenchymal cell components of injury in the kidney. Because of their interacting nature during both ischemia/reperfusion and many forms of nephrotoxic injury (125), it can be difficult to distinguish among intrinsic cell-specific, inflammatory cell, vascular cell, and tubule cell effects of protective influences that derive from preconditioning and other influences that can affect more than one cell type. There have been some informative efforts to do this rigorously within the limits of the available models (126), but that has not been the case generally. Moreover, studies usually report only work testing single maneuvers under conditions where the severity of injury is optimal for illustrating efficacy in the species and strains of animals used. The effect may or may not be generalizable, a fact that can further constrain interpretation.

Actions of protective influences on parenchymal cells may be on the endothelial or epithelial cell or both. Demonstration of effects on proximal tubules doesn’t mean they are necessarily the only or even the primary site of action. Induction of heme oxygenase during ischemia/reperfusion is an example of this in that highest levels of the enzyme appear in cortical proximal tubules, but global tubule cell protection in the outer medulla occurs (116). This makes it likely that heme oxygenase in the endothelium (127) accounts for much or all of the protection in that setting. Effects of dopamine on endothelial heme oxygenase and adhesion molecule expression may account for a protective effect in animals (128). Opening of myocardial mitochondrial K$_{ATP}$ channels by direct pharmacologic modulators, and as a result of upstream processes such as protein kinase c activation during preconditioning, protects myocytes against early reperfusion injury. The protection occurs due to limitation of mitochondrial re-energization and thus prevention of calcium uptake and development of the mitochondrial permeability transition (122). However, this form of early reoxygenation injury does not occur in renal tubules (64). In fact, nonspecific K$_{ATP}$ channel blockers, which are protective for myocytes, enhance the rapid hypoxic injury to isolated proximal tubules that occurs in the absence of glycine (129). ERK1/2 activity relative to JNK activity in most settings is a mediator and/or marker of a more favorable outcome (117,118), but ERK1/2 may also be involved in promoting tubule cell injury (130).

Repair

Proximal tubules are able to undergo repair after ischemic or nephrotoxic damage. While cell death itself is not a regenerative response, epithelial cells in the process of dying may generate signals that initiate the repair response. Cytokines may play a role in determining the fate of the epithelial cells,
contribute to the generation of signals that result in neutrophil and monocyte infiltration into the tissue, and promote dedifferentiation and proliferation of epithelial cells. These cytokines may derive from the kidney tissue, epithelial and mesenchymal cells, or infiltrating cells, such as macrophages, as described previously in this review.

There is a marked increase in proliferation of the surviving proximal tubule cells in both human acute tubular necrosis (131) and animal models of ischemia/reperfusion (132). Some of these proliferating cells are derived from apparently mature proximal tubule cells, indicating that differentiated proximal tubule cells retain the ability to dedifferentiate and proliferate. Following a burst of cell proliferation, poorly differentiated regenerative cells repopulate the damaged area. These cells possess a morphologically flattened appearance with a poorly differentiated brush border and express vimentin, an embryonic marker for multipotent kidney mesenchyme, both after toxin-induced or ischemia/reperfusion injury (132). The change in the differentiated phenotype to a less differentiated one might be important for remodeling of the proximal tubule architecture. This phase of recovery recapitulates many aspects of renal development from the perspective of protein expression. A number of proteins that are expressed at high levels in the developing metanephric mesenchyme, but not expressed to the same degree in the adult kidney epithelial cell, are expressed at high levels in the recovering kidney after ischemia. Besides vimentin, another example of such a protein is the membrane adhesion molecule NCAM (133). Likewise, other proteins that are downregulated in the metanephric mesenchyme are also downregulated in the dedifferentiated recovering tubule epithelial cell. Such a protein is Kid-1, a zinc finger transcription factor (134). The last phase of the repair process is redifferentiation. By the end of this phase, most damaged tubules have regained essential function and have recovered from the damage.

Given this very active proliferative phase, many have considered the roles of growth factors in the process (135). In addition, many investigators have evaluated the role of exogenously administered growth factors in enhancing recovery (97,136). While a number of these growth factors have shown effectiveness in animals, small studies in humans have not been encouraging (135,137). A much more extensive review of the features of dedifferentiation and proliferation of surviving epithelial cells in acute renal failure has recently been written (138).

**Biomarkers**

Clinical studies designed to test therapeutic agents to prevent or treat ARF have been severely compromised by the absence of reliable markers of early disease. As a result, therapeutic intervention is delayed and there are no sensitive markers for monitoring effectiveness. Serum creatinine is used as an overall marker for renal function but increases in this parameter often lag well behind the initiating event in ARF. There is no equivalent of troponin which can be used as an early marker for ischemia in the heart. Biomarkers for renal ischemic injury could be monitored in the blood or urine. While several urinary proteins have been evaluated as potential non-invasive markers of renal injury (139) none of these markers have been used successfully to screen for early renal injury or to identify the site of injury within the kidney.

Using a genomic approach, kidney injury molecule 1 (KIM-1) was cloned from the post-ischemic kidney (140). Kim-1 is a type 1 transmembrane glycoprotein that is upregulated in post-ischemic regenerating rat kidney. Recent studies have also found it to be a sensitive indicator for three forms of ARF due to toxins, including cisplatin, folic acid, and TFEC (Ichimura, Bonventre et al., submitted for publication). In addition, it is expressed on the proximal tubule epithelial cells in human kidney biopsy sections from patients with acute tubular necrosis (141). Importantly, there is no expression of KIM-1 in normal human kidney, consistent with the conclusion that KIM-1 is a marker of injured renal tissue. The ectodomain of human KIM-1 is detected at higher levels in the urine of patients with ischemic acute renal failure as compared with patients with other forms of acute renal failure or chronic renal disease (Figure 2). Following aortic crossclamping to a patient with mild tubular injury, urinary KIM-1 levels increase before the appearance of urinary casts, and then return to baseline. This suggests that KIM-1 may be useful as a marker of ischemic ARF early in its course, before other markers become evident. There was extensive expression of KIM-1 in proximal tubule cells in biopsies from 6 of 6 patients with confirmed ischemic ARF. In contrast, concentrations of other urinary biomarkers, including total protein, gamma-glutamyltransferase, and alkaline phosphatase, did not correlate with clinical diagnostic groupings.

**Conclusions**

Renal injury associated with ischemia/reperfusion results from a dynamic process involving the vasculature and tubules.

**Comparison of urinary KIM-1 concentration in various forms of renal diseases**

![Figure 2. Selectivity of urinary KIM-1 excretion during human renal disease. Bars indicate means ± SEM for the indicated number of patients studied under each condition. Figure and data reprinted with permission from reference 141.](image-url)
in a complex interaction whereby events modulating the vasculature will alter oxygen and nutrient delivery to the epithelial cell and the injured epithelial cell will respond by producing autocrine factors that will affect its own survival and paracrine factors which affect the vasculature. There is a complex activation of signaling cascades resulting in hemodynamic alterations, leukocyte accumulation, and direct injury to the tubule epithelial cells followed by a repair process that can restore normal morphology and function. Although significant progress has been made in defining the major components of this process, interventions that have proven effective in animal models have failed to show beneficial effect in human trials of ATN. This is not necessarily the fault of the models, although we agree they can be improved upon, but we are woefully lacking diagnostic studies to derive the important information about human acute renal failure that will help us establish the diagnosis early enough and treat ARF vigorously enough at an early enough stage and in a large enough group of patients so that we may feel confident that a proposed therapy has been adequately tested. A better understanding of the pathophysiology underlying the functional defects found in ischemic acute renal failure will also require that we take into account the complexity of illness (e.g., sepsis) in which this syndrome occurs. Clearly, while there has been significant advancement in our understanding over the recent past, much needs to be done before we can comfortably predict and adequately test therapies that will be most effective in humans.

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