

Transitioning to Therapy in Ischemic Acute Renal Failure

BRUCE A. MOLITORIS

Division of Nephrology, Department of Medicine, Indiana University School of Medicine, and the Roudebush VA Medical Center, Indianapolis, Indiana.

Ischemic acute renal failure (ARF) remains an area of immense clinical importance and cost. Outstanding advances in understanding the cellular and molecular aspects have occurred, yet little progress has been made in the translation of these findings to the clinical arena. In this issue of *JASN*, two important articles provide additional contributions to our understanding of the cellular events that mediate injury and subsequent cell death in renal tubule epithelial cells. Before discussing these articles individually, the context of their potential clinical importance should be outlined. Figure 1 illustrates the different stages of ischemic ARF and how therapy should be envisioned for each of these stages. Patients at high risk for ischemic ARF include those with a reduced effective arterial volume resulting in prerenal azotemia. Numerous ischemic insults, alone or in synergistic combination with nephrotoxins, initiate epithelial and vascular cell injury, resulting in an extremely rapid decrease in GFR appropriately termed the “initiation phase.” The initiation phase is immediately followed by a phase that has recently been termed the “extension phase” (1). During the extension phase, multiple interrelated events lead to a worsening of epithelial and endothelial cell injury and cell death, primarily in the cortical-medullary region of the kidney. Existence of this phase has been postulated to occur in the clinical arena, but substantiating physiologic data are lacking. Nevertheless this concept is built on sound investigational data from both clinical sources and animal models. It also provides an important instructional framework upon which to base therapy, and it emphasizes the absolute necessity of a rapid diagnosis and institution of therapy in clinical ARF. The “maintenance phase” represents a phase of stabilization of injury, and subsequent correcting events leading to cellular repair, division, and redifferentiation. This sets the stage for improved epithelial and endothelial cell function and recovery of GFR during the “recovery phase.”

Therapy for ARF must be considered in the context of these different phases. Utilizing this approach, it is easy to understand why studies that initiate therapy during the maintenance phase have proven uniformly unsuccessful. On the other hand, significant progress has been made in the prevention of ischemic ARF, especially in regard to the high-risk population,

defined as having a reduced effective arterial volume or underlying renal disease. It is also essential to view the importance of our understanding of the basic science of ischemic ARF in the context of these different phases. For example, studies involving preconditioning are trying to limit the extent of initial cell injury, thereby minimizing the severity of both the initiation and extension phases. Classically, two phases of preconditioning have been described and consist of an immediate phase and a delayed phase. Unfortunately, only the delayed phase has been substantiated in the kidney. Heat shock proteins represent potential mediators of preconditioning effects. Previous work in renal epithelial cells has clearly shown that Hsp 70 is induced by renal ischemia and that both the cytosolic and ER forms can be protective (2–4). HSP-25/27 is another class of heat shock proteins known also to be involved in actin dynamics by being barbed end-capping proteins. They have been shown to protect the actin cytoskeleton during oxidative stress, and they interact with the actin cytoskeleton during and after renal ischemia (5–7). Actin cytoskeletal alterations occur early and in a duration-dependent fashion during ischemic cell injury. They play an important role in mediating alterations in cell-cell attachment, cell-ECM attachment, microvillar collapse, and loss of epithelial polarity (8). To determine the potential protective effect of the small heat shock proteins Hsp25/27, Van Why *et al.* (9) utilized expression of the fusion protein HSP 27-GFP to specifically track the intracellular movements and associations of this group of proteins with actin under physiologic conditions, during ATP depletion and during cellular recovery of ATP. They used fluorescence resonance energy transfer (FRET) to firmly document the close association between Hsp 27-GFP and rhodamine phalloidin-stained F actin. This is an extremely specific technique and was necessary, as the diffuse localization of Hsp 27-GFP throughout the cell could result in apparent co-localization without true molecular associations. The authors then used the HSP 27-GFP probe and assumed that endogenous Hsp 25 behaved similarly. This is probably a reasonable assumption, given previous work by this group showing association of Hsp25 with rhodamine phalloidin-labeled F actin (5). Notable association between the Hsp 27-GFP and rhodamine phalloidin staining occurred throughout the cell in cytosolic aggregates and along the lateral aspects of the cell, where densely stained deposits were seen. These investigators went on to show that Hsp 27 expression reduced ATP depletion-induced dissociation of Na⁺,K⁺-ATPase from the cytoskeleton. These data support and extend observations of several recent articles utilizing GFP technology in renal epithelial cells to determine the effect of ATP depletion on the actin cytoskeleton. Herget-Rosenthal *et al.* (10)

Correspondence to Dr. Bruce A. Molitoris, Division of Nephrology/ Department of Medicine, 1120 South Drive, Fesler Hall 115, Indianapolis, IN 46202. Phone: 317-274-7453; Fax: 317-274-8575; E-mail: bmolitor@iupui.edu

1046-6673/1401-0265

Journal of the American Society of Nephrology

Copyright © 2003 by the American Society of Nephrology

DOI: 10.1097/01.ASN.0000048852.53881.D9

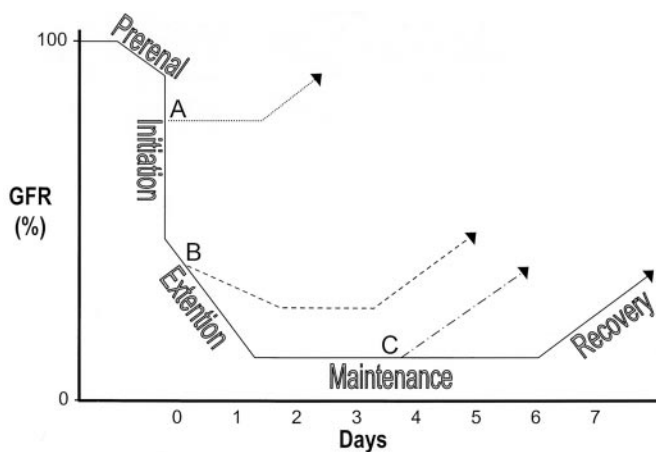


Figure 1. Phases of ischemic acute renal failure. A, B, and C refer to therapies aimed at preventing (A); limiting the extension phase (B); and treating established ARF (C).

used EYFP-actin to visualize actin dynamics during ATP depletion and repletion in LLC-PK₁₀ cells, and Sheldon *et al.* (11) used EGFP-actin to evaluate site-specific alterations in actin assembly during cellular ATP depletion in LLC-PK1 cells. Taken together, these studies confirm the rapid breakdown of cortical and stress fiber components of the actin cytoskeleton and site-specific enhanced actin polymerization at sites of epithelial cell-cell attachment and in cytosolic aggregates. Sheldon *et al.* (7) have also previously shown Hsp 27 associated with the lateral cell boundaries in ATP-depleted epithelial cells. The present work therefore completes a circle showing that during ATP depletion there is assembly of F actin in association with Hsp 27 in the cytosol and at sites of cell-to-cell contact. The assembly of actin at these sites begins rapidly after the start of ATP depletion, while other sites of cellular F actin, such as the stress fibers, are being depolymerized. Is there a functional significance to assembly at cell-cell junctions during ATP depletion, or is this merely a consequence of the concentration of G actin and regulatory proteins at this point? Cell-cell dissociation clearly occurs during ATP depletion, even though assembly of the actin cytoskeleton at these points is occurring. The fact that Hsp 27 upregulation leads to stabilization of Na⁺,K⁺-ATPase with the actin cytoskeleton is suggestive of a functional role of Hsp 27 in minimizing actin cytoskeletal alterations during ATP depletion. However, whether this has an effect on cell-cell attachment, cell-cell extracellular matrix attachment, cell repair, or cell viability through either necrosis or apoptosis remains to be determined.

The role of apoptosis in ischemic ARF has been a controversial area. Although known to occur, quantifying apoptosis *in vivo* is difficult because it is an ongoing process, with apoptotic cells appearing and disappearing in a relatively short period of time. Therefore, the extent of apoptotic cell injury relates to an integrated area under the curve requiring repeated observations over a prolonged period of time. Even more challenging is quantifying the role of apoptosis in the reduction of renal function after ischemic injury. In this issue of *JASN*,

the work of Kelly *et al.* (12) emphasizes the potential importance of apoptosis in ischemic ARF. Using a moderate level of ischemic injury, 30 min of clamp ischemia in the rat model, the authors showed that expression of p53 in the cortical-medullary area increased over 24 h, while cortical p53 increased but then rapidly decreased to baseline values. Treatment with guanosine, previously shown by these authors to be protective, increased cellular GTP, prevented p53 upregulation, reduced apoptosis, minimized reductions in GFR, but had no effect on overall cellular histology. To more thoroughly evaluate the selective role of apoptosis in mediating the decrease in GFR, Kelly *et al.* (12) used a selective inhibitor of p53 function. Pifithrin- α , by binding to p53, inhibits mitochondrial and nuclear localization, thereby limiting its potential effects on downstream mediators of apoptosis, including p21 and Bax. Therefore, an increase in cellular p53, secondary to GTP depletion, or any of the other known inducers, can be effectively neutralized. These include genotoxic stresses mediating DNA damage that also lead to phosphorylation and activation of p53 (13,14).

The present data imply, but do not prove, that intracellular depletion of GTP may directly initiate the apoptotic cascade via p53 activation independent of the inflammatory cascade. Additional evidence supports this concept in endothelial cells (15). Therefore, multiple pathways induce p53 during ischemic/hypoxic injury. Having the ability to neutralize the function of p53 means therapeutic agents can minimize the effects of multiple intracellular cascades.

Another remarkable aspect of this article relates to the effectiveness of pifithrin- α after extended periods of reperfusion. When administered even 14 h after ischemic injury, the agent was still partially effective. It was also effective in the cortical-medullary area, an area not well reperfused after ischemic injury (1). Lack of blood flow to the cortical-medullary area during reperfusion results in continued hypoxia and further cell injury. It also minimizes drug delivery to this critical area, limiting our ability to therapeutically approach the extension phase of ARF.

Multiple questions remain to be answered regarding p53 blockade using pifithrin- α . For instance, is the effect seen for both epithelial and endothelial cells? Does it effect phosphorylation of p53 or the essential interaction between p53 and Pin1 (16)? Will short-term inhibition of apoptosis result in an increased tendency toward a malignant phenotype in cells or enhanced inflammation by limiting apoptosis in other cell types? Furthermore, does prevention of apoptosis in the initial stage of reperfusion result in a sustained protection of GFR? Or do these “protected” cells undergo apoptosis and/or necrosis later, resulting in an enhanced fibrotic response?

The present data also represent the second time these authors have dissociated cellular morphology and function. The data imply the reduction in GFR seen after ischemic injury is more dependent on ongoing injury or other cellular events than the initial epithelial and vascular cell injury. Does this relate to increased reperfusion and enhanced GFR based primarily on hemodynamic factors?

In all therapeutic studies of ischemic ARF, it is important to

differentiate between direct and indirect effects of the agent in question. As ischemic ARF is a multifactorial process with numerous interrelated cascades, direct prevention of a proximal event will result in an effect on downstream processes. For example, multiple growth factors are known to be protective when given before, during, and even shortly after the ischemic event in animal models. These factors are known to have pleiotropic effects, with one effect being to increase renal blood flow. If this in turn increases cortical-medullary blood flow, then the observed decrease in apoptosis, inflammatory cell infiltrate, and oxygen free radical formation may well be indirect effects of increased perfusion and not due directly to the therapeutic agent. However, preventing or limiting these intracellular cascades is indeed the goal of preventative therapy and therapy given during the extension phase of ARF (Figure 1). It underscores the necessity of early diagnosis and rapid therapy in ARF, an area of little attention and progress in nephrology. Just as identifying patients at high risk for ARF has minimized the incidence and extent of ARF, so will developing approaches for the early diagnosis and rapid treatment of ischemic ARF.

References

1. Sutton TA, Fisher CJ, Molitoris BA: Microvascular endothelial injury and dysfunction during ischemic acute renal failure. *Kidney Int* 62: 1539–1549, 2002
2. Bush KT, George SK, Zhang PL, Nigam SK: Pretreatment with inducers of ER molecular chaperones protects epithelial cells subjected to ATP depletion. *Am J Physiol* 277: F211–F218, 1999
3. Li F, Mao HP, Ruchalski KL, Wang YH, Choy W, Schwartz JH, Borkan SC: Heat stress prevents mitochondrial injury in ATP-depleted renal epithelial cells. *Am J Physiol Cell Physiol* 283: C917–C926, 2002
4. Turman MA, Rosenfeld SL: Heat shock protein 70 overexpression protects LLC-PK1 tubular cells from heat shock but not hypoxia. *Kidney Int* 55: 189–197, 1999
5. Aufricht C, Ardito T, Thulin G, Kashgarian M, Siegel NJ, Van Why SK: Heat-shock protein 25 induction and redistribution during actin reorganization after renal ischemia. *Am J Physiol* 274: F215–F222, 1998
6. Ehrnsperger M, Graber S, Gaestel M, Buchner J: Binding of non-native protein to Hsp25 during heat shock creates a reservoir of folding intermediates for reactivation. *Embo J* 16: 221–229, 1997
7. Sheldon EA, Borrelli MJ, Pollock FM, Bonham R: Heat shock protein 27 associates with basolateral cell boundaries in heat-shocked and ATP-depleted epithelial cells. *J Am Soc Nephrol* 13: 332–341, 2002
8. Atkinson SJ, Molitoris BA: Cytoskeletal alterations as a basis of cellular injury in acute renal failure. In: *A Companion to Brenner & Rector's The Kidney*, edited by Molitoris BA, Finn WF, Philadelphia, WB Saunders, 2001, pp 119–131
9. Van Why SK, Mann AS, Ardito T, Thulin G, Ferris S, MacLeod MA, Kashgarian M, Siegel NJ: Hsp27 associates with actin and limits injury in energy depleted renal epithelia. *J Am Soc Nephrol* 14: 98–106, 2002
10. Herget-Rosenthal S, Hosford M, Kribben A, Atkinson SJ, Sandoval RM, Molitoris BA: Characteristics of EYFP-actin and visualization of actin dynamics during ATP depletion and repletion. *Am J Physiol Cell Physiol* 281: C1858–C1870, 2001
11. Sheldon EA, Weinberg JM, Sorenson DR, Edwards CA, Pollock FM: Site-specific alteration of actin assembly visualized in living renal epithelial cells during ATP depletion. *J Am Soc Nephrol* 13: 2667–2680, 2002
12. Kelley KJ, Plotkin Z, Vulgamott SL, Dagher PC: P53 mediates the apoptotic response to GTP depletion after renal ischemia-reperfusion: protective role of a p53 inhibitor. *J Am Soc Nephrol* 14: 128–138, 2002
13. Bates S, Vousden KH: Mechanisms of p53-mediated apoptosis. *Cell Mol Life Sci* 55: 28–37, 1999
14. Vogelstein B, Lane D, Levine AJ: Surfing the p53 network. *Nature* 408: 307–310, 2000
15. Stempien-Otero A, Karsan A, Cornejo CJ, Xiang H, Eunson T, Morrison RS, Kay M, Winn R, Harlan J: Mechanisms of hypoxia-induced endothelial cell death. Role of p53 in apoptosis. *J Biol Chem* 274: 8039–8045, 1999
16. Zheng H, You H, Zhou XZ, Murray SA, Uchida T, Wulf G, Gu L, Tang X, Lu KP, Xiao ZX: The prolyl isomerase Pin1 is a regulator of p53 in genotoxic response. *Nature* 419: 849–853, 2002

See related articles, “Hsp27 Associates with Actin and Limits Injury in Energy Depleted Renal Epithelia” (pp. 98–106) and “P53 Mediates the Apoptotic Response to GTP Depletion after Renal Ischemia Reperfusion: Protective Role of a p53 Inhibitor (pp. 128–138).