Therapeutic Targets of Human AKI: Harmonizing Human and Animal AKI

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ABSTRACT

The opportunity to make advances in the prevention and treatment of AKI has never been greater than it is today. Major advances have been made in the understanding of the biology of AKI, the design of clinical trials, and the use of diagnostic and prognostic biomarkers. These advances have been supplemented by the coordinated effort of societies, federal agencies, and industry, such that we are poised in the ensuing years to positively address the unrelenting harm that this disorder has created. Over the past decade, major advances have been made in understanding the pathophysiology of AKI, mainly through the study of small animal models. However, translating these findings to human AKI remains a barrier, which is typified by the absence of effective therapeutic agents. The purpose of the Acute Dialysis Quality Initiative (ADQI) XIII was to harmonize human and animal studies and determine what is known about potential therapeutic targets and what gaps in knowledge remain. A series of invited reviews will distill key concepts from this initiative that focus on different pathogenic features of AKI, including hemodynamics, immunity and inflammation, cellular and molecular pathways, progression, and regeneration and repair. This series will convey the status of our knowledge of the pathophysiology of human AKI and propose therapeutic targets for further investigation.


AKI is a growing public health burden. The incidence of AKI steadily rises, leading to high morbidity, high mortality, and increased health care costs through hospitalizations and development of CKD and ESRD. In September of 2000, the National Institutes of Health (NIH) sponsored a meeting entitled “Design Issues for Clinical Trials in Acute Renal Failure,” which was focused on overcoming barriers to improving outcomes in AKI.1,2 Subsequently, a prolific decade and a half ensued, with research focusing on biomarkers of AKI, novel drug targets, developing new clinical disease definitions, and clinical trial designs. Despite these advances, our knowledge is incomplete, and effective therapies are lacking.3 An important gap in our knowledge is extrapolating information from animal models of AKI to human AKI. To advance the field, harmonizing animal models of AKI and human AKI is critical in identifying meaningful targets for drug development and testing in human clinical trials. To achieve these goals, the Acute Dialysis Quality Initiative (ADQI) XIII meeting was designed to assemble stakeholders in AKI, including lead investigators in AKI, industry partners, the NIH/US Food and Drug Administration (FDA), and the Kidney Health Initiative (KHI). The KHI is a partnership between the American Society of Nephrology and the FDA. This public-private partnership with the kidney community seeks to understand and promote kidney health and patient safety. This group was asked to critically analyze the data available in the current literature and develop up-to-date understanding of the pathophysiology of AKI on the basis of the methods used previously from prior ADQI conferences (Supplemental Appendix 1). In addition, focus was placed on the relevance and translation of animal studies to human AKI. This latter issue is of high relevance, because potential therapies identified in animal models for human disease have often failed when subsequently tested in humans.4

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HUMAN AKI

Therapeutic failures in clinical studies of AKI have reinvigorated questions regarding clinical trial design and whether animal models of AKI can inform human disease. Clinical trials have been hampered by many shortcomings that include factors, such as inadequately powered studies, the masking of potential benefit because of adverse events, and the late timing of intervention. Radiocontrast-induced AKI has been the focus of many prevention studies since an early report of the success in the prevention of AKI by N-acetyl cysteine. Since then, numerous follow-up studies have been performed that questioned the benefit of N-acetyl cysteine in the prevention of radiocontrast-induced AKI, leaving clinicians with uncertainty as to the benefit of this drug. Other preventative and therapeutic studies have continued to provide variable results that stem from inadequately powered and poorly designed studies. Other potential benefits of a particular intervention may have been concealed by adverse events. For example, atrial natriuretic peptide was used in an interventional study in critically ill patients and had no significant effect on dialysis-free survival, the primary end point. The potential benefit of atrial natriuretic peptide may not have been detected because of the more frequent episodes of hypotension that occurred in the treatment arm. In most animal studies, early intervention has been determined to be critically important and by the nature of the experimental design, can be ensured. In human clinical trials, the lack of efficacy may have been because of the late timing of intervention. For example, in a multicenter study examining the efficacy of recombinant IGF-1 in critically ill patients, the lack of efficacy may be, in part, because of the administration of the hormone as late as 6 days after the onset of AKI. Thus, a critical need is to improve the design and conduct of clinical trials in AKI, maximizing the potential identification of new drugs in the prevention and treatment of AKI.

ANIMAL MODELS OF AKI

In addition to shortcomings of clinical trial design, animal models are often deemed poor predictors of experimental drug effectiveness. The failure of experimental models may be caused by methodological flaws, or these models may not closely mimic the human disease that forms the basis of a subsequent interventional clinical study. Animal models tend to use very stereotypical insults, such as renal artery clamping or cecal perforation, to mimic clinical states that are likely much more complex, such as ischemia-reperfusion associated with cardiac surgery or septic shock. Publication bias of only successful studies may lead to erroneous conclusions regarding the efficacy of a specific therapeutic intervention. Many preclinical AKI studies are performed in young male mice of a single genetic strain with normal renal function. However, human AKI often occurs in older subjects of both sexes who have concomitant comorbidities, including CKD.

Much discussion has been centered on the development of more robust models of AKI that closely mimic human disease and include such common comorbidities as diabetes mellitus, obesity, vascular disease, and atherosclerosis. However, whether such complex models will be game changers is uncertain, and they will undoubtedly be costly, be time consuming, and introduce a multitude of additional confounding variables. This leads to central questions. What is the purpose of animal studies? To what extent do animal models need to mimic all aspects of human disease? How should other experimental platforms be studied, such as zebrafish? For instance, there are many advantages of zebrafish, including the fact that humans share 70% of their genome with zebrafish and that the majority of these are linked to human disease. They allow for molecular targeting and rapid in vivo drug-screening studies. Certainly, mouse studies offer the array of genetic manipulations to precisely define molecular targets of AKI. These models combined with targeted pharmacology can powerfully define whether a certain molecular target is responsible for a desired effect and serve as a target for therapy. The use of several models of AKI might allow for determination of generalizability to other forms of AKI, including large animals. For example, if an endothelial target is important in ischemia-reperfusion injury, would this same target be important in sepsis or nephrotoxic injury? Importantly, models for AKI should recapitulate the clinical disease. Sepsis, cardiac surgery, and transplant can be induced in animals, and these clinical conditions are not modeled by prolonged warm ischemia-reperfusion.

TRANSITION FROM ANIMAL TO HUMAN STUDIES

Although there has been an explosion in the identification of potential drug targets through information gathered through molecular, genetic, cellular, and physiologic processes, the translation to human studies has been largely ineffective for many of the reasons discussed. However, other factors often ignored are important in transition from animal to human studies. The efficacies of drug therapy, which have been identified in animal studies, when applied in human clinical trials depend on the extent to which the therapy binds to their cognate targets. It is important to determine the specificity, because off-target binding can lead to adverse side effects. Molecular targets in animal studies need to be confirmed and validated in human studies. Additional questions need to be addressed. Are molecular targets identified in animals conserved across species? Do these orthologous genes express target protein in similar locations as in animal studies? Do these conserved proteins share similar pharmacologic and pharmacodynamic properties in humans and animals? Is there evidence in vitro and in vivo of ligand-target engagement? Lastly, what are the critical local drug concentrations in the microenvironment that dictate efficacy? Studies addressing these questions remain critical in the transition from...
animal studies to clinical trials. The current failure to transition to human studies remains a major concern in our search to find cures for AKI. Because the cost of clinical trials is so high, the importance of robust methods to ensure that targets identified in animal models are suitable human targets is critical, and this was the focus of ADQI XIII and its five workgroups.

Each of the workgroups reviewed the literature on recent advances in the pathogenesis of AKI and where available, information concerning human AKI. The consensus opinions are on the basis of synthesizing this information and defining gaps in our knowledge that require additional study. A series of invited reviews will distill key concepts by the five workgroups, including hemodynamics, immunity and inflammation, cellular and molecular, progression, and regeneration and repair. The first in the series, hemodynamics, appears in this issue of the Journal of the American Society of Nephrology.

HEMODYNAMICS (WORKGROUP 1)

The concept of controlling global renal blood flow has served as the basis for the prevention and treatment of AKI. When blood flow is decreased below the autoregulatory threshold, glomerular filtration decreases with resulting AKI. However, enhancing blood flow through the use of vasodilators, such as dopamine, fenoldopam, atrial natriuretic peptide, or calcium antagonists, in clinical studies has not been shown to be beneficial.15 Although complete cessation of renal blood flow is associated with AKI in animals and humans, in experimental animals, 80% reduction of renal blood flow for 2 hours is transiently associated with a decrease in renal function; however, recovery is rapid, and there is no morphologic evidence of acute tubular necrosis 5 days after occlusion.16 Thus, despite significant hypoperfusion, there was minimal injury. Workgroup 1 emphasizes that the heterogeneity of microcirculatory blood flow is critical in determining the pathogenesis of AKI. Local factors, such as molecules of tissue inflammation, reactive oxygen species, and other factors that control microcirculatory response to injury, as well as the endothelial glycocalyx are discussed by this workgroup along with additional key topics for investigation.

IMMUNITY AND INFLAMMATION (WORKGROUP 2)

Workgroup 2 focused on the role of immunity and inflammation. Experimental studies have shown a critical role of innate immunity in the pathogenesis of AKI. Early release of intracellular mediators referred to as damage-associated molecular patterns or reactive oxygen species initiates a cascade of events, leading to early activation of antigen-presenting cells, such as dendritic cells and macrophages, and innate immunity. Important roles of dendritic cells, macrophages, T and B cells, natural killer T cells, and neutrophils in the pathogenesis of AKI have been shown, whereas other studies have shown that regulatory T cells, dendritic cells, and natural killer T cells are protective in AKI. However, it should be said that most of this work has been in rodent models of AKI. Very little work on leukocytes in AKI has been studied in humans. In rodent studies, various models have shown the importance of innate immunity, including ischemia-reperfusion, cisplatin, sepsis, and aminoglycoside toxicity. Workgroup 2 focuses on the mechanism that innate immunity plays in the generation and maintenance of AKI.

CELLULAR AND MOLECULAR (WORKGROUP 3)

Workgroup 3 discussed the role of cellular and molecular mechanisms that govern normal cellular function and when disrupted, lead to AKI, and they could serve as targets for therapeutics. Epithelial cells undergo apoptosis and necrosis, leading to tubule cell obstruction and back leak of glomerular filtrate in AKI. Epithelial cells undergo self-renewal through a carefully synchronized sequence of events that leads to genomic DNA replication and cell proliferation.30 Cell cycle regulatory proteins (cyclins and cyclin-dependent kinase subunits) are synthesized and degraded during the cell cycle, which allows the cycle to proceed in one direction. Normally kidney epithelial cells are quiescent at the G0 phase of cell cycle; however, after AKI, there is cellular proliferation and cellular reactivation. Much is known about the mechanism regulating the cell cycle, and recently, two important molecules, inhibitor of metalloproteinases-2 and IGF-binding protein 7, have been identified in cell cycle arrest and shown to be early markers of AKI.31 What remains to be seen is whether they can serve as important targets for therapeutics as well as diagnostics in AKI through preventing damaged DNA from entry into the cell cycle as well as conserving energy during injury. Critical cellular energy is derived from mitochondrial activity. Recent advances in the study of mitochondrial biogenesis and mitophagy as well as the dynamic balance with fusion and fission that governs efficient ATP generation and susceptibility to apoptotic stimuli are discussed by workgroup 3.

PROGRESSION (WORKGROUP 4)

Widely recognized is the association between small increases in creatinine and increased mortality.33,34 Additionally, observational studies have drawn an association with progression of kidney disease, leading to ESRD in patients who survive an initial bout of AKI.35–39 The risk for CKD and ESRD is significant after AKI. Ishani et al.38 found in a cohort of Medicare beneficiaries who were hospitalized and survived AKI that the risk for ESRD was 41.2% for those with AKI and CKD and 13% for those with AKI alone. Despite these epidemiologic data, the underlying mechanisms that explain the progression of kidney disease after AKI are incompletely understood.
Maladaptive process that govern the progression of kidney disease include endothelial injury, tissue hypoxia, capillary rarefaction, interstitial fibrosis initiated by pericyte activation, epithelial-mesenchymal transition, and others. Molecules, such as hypoxia-inducible factor, vascular endothelial growth factor, and nuclear factor erythroid 2–related factor 2 activation, are thought to contribute to progressive fibrosis. Workgroup 4 reviewed various animal models to study progression after AKI.

REGENERATION AND STEM CELLS (WORKGROUP 5)

The course of AKI is well described by Molitoris and Sutton and Sutton et al. After reduction of blood flow, there is initial cellular injury and decline in GFR during the initiation phase. Activation of the innate immune system leads to additional injury and decline in GFR during the extension phase of AKI. The ensuing phase heralds the recovery from AKI in those kidneys destined for recovery. During the maintenance phase, cellular repair processes are initiated, and organ integrity is re-established; during the recovery phase, return of organ function follows with improvement in GFR. A critical concept in the regenerative process is what actually has recovered. GFR as assessed by serum creatinine is generally used to measure return of renal function, but a much broader definition is necessary. Workgroup 5 introduces the concept of complete renal function repair, which provides an assessment of degree of recovery incorporating renal perfusion, GFR, and tubule function. The use of genetically modified mice that permit fate mapping has shed new light on the mechanism of tubule regeneration. Recent advances highlight the potential for cell-based therapies; however, recent failure of mesenchymal stem cell–based therapy for AKI highlights the need to understand gaps in our knowledge in transitioning from animal to human studies.

In summary, over the past two decades, much progress has been made in understanding the pathogenesis, progression, and repair of AKI. Drug discovery, using a number of different platforms, has defined novel compounds tested in human clinical trials. The combined efforts of a number of stakeholders, including academic investigators, industry, the NIH, the FDA, and more recently, the KHI, have been critical in the efforts to date. In the future, harmonizing our understanding from preclinical studies in AKI and implementing this knowledge in well-designed human clinical trials will be critical. Importantly, understanding similarities and differences in human and animal AKI will necessitate rigorous studies of human and animal molecular targets, translational studies to understand drug concentrations in the tissue microenvironment, and careful pharmacokinetic and pharmacodynamic studies in humans as well as demonstration of ligand-target engagement. These efforts will hopefully lead to major advances in improving outcomes in AKI.

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REFERENCES


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