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Pathogenesis of Acute Kidney Injury: Foundation for Clinical Practice

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Abstract

The pathogenesis of acute kidney injury (AKI) is complex, involving factors such as vasoconstriction, leukostasis, vascular congestion, cell death, and abnormal immune modulators and growth factors. Many targeted clinical therapies have failed, are inconclusive, or have yet to be tested. Given the complexity of the pathogenesis of AKI, it may be naïve to expect one therapeutic intervention would have success. Some examples of detrimental processes that can be blocked in pre-clinical models to improve kidney function and survival are apoptotic cell death in tubular epithelial cells, complement-mediated immune system activation, and impairment of cellular homeostasis and metabolism. Modalities with potential to reduce morbidity and mortality in AKI include vasodilators, growth factors, anti-inflammatory agents, and cell-based therapies. Pharmacological agents that target these diverse pathways are being used clinically for other indications. Using combinatorial approaches in future clinical trials may improve our ability to prevent and treat AKI.

Keywords

Acute renal failure; inflammation; acute tubular necrosis; hemoglobinuria

BACKGROUND

The pathogenesis of acute kidney injury (AKI) is complex. Whereas initiating events may be dissimilar (ischemia or toxins are major factors that precipitate injury), subsequent injury responses may involve similar pathways. The complexity of AKI is illustrated in the following example. AKI associated with ischemia from a reduction of renal blood flow below the limits of auto-regulation leads to maladaptive molecular and cellular responses. These responses cause endothelial and epithelial cell injury following the onset of reperfusion (1). Pathogenic factors such as vasoconstriction, leukostasis, vascular congestion, cell death, and abnormal immune modulators and growth factors have formed the basis of rational therapeutic interventions (2-6). However, many of these targeted clinical therapies have failed, are inconclusive, or have yet to be tested (7, 8). Given the

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complexity of the pathogenesis of AKI, it may be naïve to expect one therapeutic intervention would have success unless that intervention focuses on prevention of AKI and targets a specific initiating event. Given the multiple overlapping pathways involved in AKI, therapies may need to simultaneously target multiple pathways in order to achieve success(9). This review will highlight several pathogenic mechanisms in AKI where pre-clinical studies have demonstrated novel therapeutic targets for combination therapies (Box 1).

CASE VIGNETTE

A 48-year-old Caucasian female with non-Hodgkin lymphoma presented with sudden onset of fever, chills, and myalgias. Approximately 1 week prior to admission she received methotrexate and cytosine arabinoside as part of Hyper-CVAD (cyclophosphamide, vincristine, doxorubicin) regimen for mantle cell lymphoma. Medications included acyclovir and granulocyte colony-stimulating factor (G-CSF). On examination she was well-developed and well-nourished, but in moderate distress. Vital signs included temperature 38.7°C, blood pressure 108/54 mmHg and pulse 132 beats/min while supine, and blood pressure 96/57 mmHg and pulse 140 beats/min while sitting. Her sclera was anicteric, there was no jugular venous distention, the lung fields were clear, and heart sounds and abdominal exam were normal. Initial laboratory tests included serum urea nitrogen 12 mg/dl (4.2 mmol/L), creatinine 0.7 mg/dl (62 µmol/L), eGFR 89 ml/min/1.73 m² (1.48 mL/s/1.73 m²), Hct 27.1%, WBC $0.1 \times 10^3/\mu\text{L}$ ($0.1 \times 10^9/\text{L}$), and platelet count $0.034 \times 10^3/\mu\text{L}$ ($0.034 \times 10^9/\text{L}$). Chest x-ray was normal and urinalysis revealed specific gravity 1.015, trace blood, 4 RBCs per high-power field, 2 WBCs per high-power field and moderate bacteria. Blood and urine samples were sent for culture. Soon after admission, she was transferred to the medical ICU after developing jaundice and dark urine, and her blood pressure fell to 80/60 mmHg. Intravenous cefepime and gentamicin were given, and acyclovir was continued. Blood was centrifuged demonstrating hemolysis and a urine sample collected 6 hrs later showed large blood (Figure 1). Blood work at that time showed a marked drop in hematocrit to 10.9%, WBC, $0.06 \times 10^3/\mu\text{L}$ ($0.06 \times 10^9/\text{L}$), platelet count $0.029 \times 10^3/\mu\text{L}$ ($0.029 \times 10^9/\text{L}$), total bilirubin was 11.5 mg/dl (197 µmol/L), AST 1068 U/L, LDH 5964 U/L, PT 16.6 sec, PTT 38.8 sec, D-dimers 2000-4000 and fibrinogen 272 mg/dl (8.0 µmol/L). Blood cultures grew gram-positive rods, and metronidazole and vancomycin were added. The patient was transfused packed red blood cells (RBC), platelets, and fresh frozen plasma. Plasma creatinine increased daily to 1.7 mg/dl (150.3 µmol/L) and 3.4 mg/dl (300.6 µmol/L). Repeat urinalysis showed muddy brown casts and numerous RBCs per high-power field. The patient was diagnosed with nonoliguric AKI secondary to sepsis, hemoglobinuria, and hypotension. Intravenous bicarbonate was administered and the creatinine peaked at 3.4 mg/dL (300.6 µmol/L) with urine output of 11 L/day. Blood cultures grew *Clostridium perfringens*.

PATHOGENESIS

This case illustrates the complexity and multifactorial nature of AKI. To date our therapies have been mostly supportive with management of fluid, electrolyte, and acid-base balance. There has been very little change in morbidity and mortality despite advances made in intensive care medicine. Many clinical trials to improve outcomes have failed forcing investigators to revisit mechanisms of AKI. A wealth of information has been gained recently and is summarized in this article.

Tubular Epithelial Cell Apoptosis

Ischemia and toxic insults to the kidney preferentially cause cell death of tubular epithelial cells, promoting decreased kidney function. The tubular epithelial cells are highly

susceptible due to 1) their high demand for oxygen and ATP (adenosine triphosphate) to drive transport functions, 2) the concentration of toxic substances inside these cells during transport into the urine, and 3) an environment of very low oxygen tension.

Apoptotic cell death is prominent in the kidney after ischemic or toxic insults(10, 11). Apoptosis is an ATP-dependent, highly regulated type of cell death that results in cell shrinkage rather than swelling observed in necrosis (12). In addition, apoptotic cells maintain plasma membrane integrity and externalize phosphatidylserine as a phagocytosis signal to macrophages or neighboring cells. Apoptosis can be initiated by extracellular stimuli or signals from inside a cell. Whereas severe ATP depletion promotes necrotic cell death, GTP (guanosine triphosphate) depletion is a key trigger for apoptotic cell death in the post-ischemic kidney (13, 14).

Extracellular-mediated or extrinsic apoptosis involves the binding of ligands to receptors on the plasma membrane (such as tumor necrosis factor [TNF]-related apoptosis-inducing ligand [TRAIL], Fas, and TNF receptor 1 [TNFR1]). Ligand binding induces receptor aggregation and formation of the death-inducing signaling complex (DISC). The DISC is composed of the activated receptor, Fas-associated death domain (FADD) and procaspase 8. The caspases are a family of cysteine proteases that facilitate initiation and execution of cell death in most forms of apoptosis. The formation of the DISC causes the cleavage of procaspase 8 to the active initiator caspase 8. Caspase 8 then cleaves and activates the executioner procaspases-3, 6 and 7 directly. In the kidney, death receptors are expressed and up-regulated after ischemic injury (15, 16), and mice lacking Fas receptors or TNF- α receptor (TNFR1) also suffer less apoptosis and decreased kidney function after cisplatin treatment(17).

Intrinsic apoptosis can be initiated through opening of the mitochondrial permeability transient pore (18), mitochondrial fragmentation (19), or permeabilization of the outer mitochondrial membrane by members of the B cell lymphoma 2 (BCL-2) family (20) causing release of cytochrome c and other factors. Mice lacking cyclophilin D (a critical component of the mitochondrial permeability transient pore) are protected from renal ischemia reperfusion injury(21). Several pro-apoptotic members of the BCL-2 family, including BID (BH3-interacting domain death agonist) and BAX (BCL2-associated X protein), translocate to mitochondria and form pores in the mitochondrial outer membrane leading to release of apoptotic mediators in animal models of AKI (20, 22, 23). On the other hand, over expression of anti-apoptotic BCL-xL (BCL-2-like protein 1) prevents cytochrome c release, apoptosis, and loss of kidney function after ischemia-reperfusion injury (24). When cytochrome c is released from the mitochondria it interacts with APAF-1 (apoptotic peptidase activating factor 1) and procaspase 9 to form the apoptosome. The apoptosome, in the presence of ATP, activates caspase 9, which then activates procaspase 3. Endonuclease G and apoptosis-inducing factor (AIF) are also released from mitochondria and participate in DNA degradation associated with apoptosis (25, 26). Exposure of tubular epithelial cells *in vitro* (27), or mice *in vivo* (26), to cisplatin leads to proteasomal degradation of the anti-apoptotic BCL-2 family member MCL-1, promoting BAX-mediated mitochondrial outer membrane permeabilization and release of AIF.

Another major mediator of apoptosis in tubular epithelial cells is the tumor suppressor protein p53 (28-32). Once activated, p53 enhances the transcription of pro-apoptotic genes including the BCL-2 protein PUMA- α (30), caspases 6 and 7 (33), PERP (p53 apoptosis effector related to PMP-22; (34)), and PIDD (p53-induced protein with a death domain). PIDD activates caspase 2 leading to mitochondrial AIF release (35). In addition, p53 activation suppresses transcription of the anti-apoptotic taurine transporter (36, 37) and hypoxia inducible factor-1 α (HIF-1 α) (38). Apart from transcriptional activity, p53 has

cytosolic and mitochondrial activity which may also contribute to tubular epithelial cell apoptosis (39).

In summary the mechanisms of renal tubular cell death involve many pro- and anti-survival factors. Pharmacological manipulation of these processes to enhance tubular epithelial cell survival may lead to reduced kidney injury and decreased function.

Immune Mechanisms of AKI

Immune mechanisms have been best studied in a model of ischemia-reperfusion injury and summarized in Figure 2. The low oxygen tension in the S3 segment of the proximal tubule located in the renal medulla contributes to the susceptibility of this segment to injury (40, 41). Innate immune leukocytes, endothelial cells, and epithelial cells contribute to early ischemia-reperfusion injury with subsequent inflammation (reviewed in^{2,3,42-44}). Although immune cell infiltration is likely to be an adaptive response to injury initiating processes involved in tissue repair, it is the overzealous, uncontrolled inflammation and breakdown of the regulation of inflammatory responses that results in tissue injury. This “collateral” damage is similar to that which occurs following the inflammatory response to infections. Resident kidney dendritic cells are the dominant resident leukocyte subset, and reside in the interstitial extracellular compartment throughout the whole kidney (45). Here they are in close apposition to epithelial cells, macrophages, and fibroblasts (46), and although their normal role in maintaining homeostatic functions is uncertain, they do respond to endogenous molecules released from resident or infiltrating cells (including lymphocytes and natural killer T cells, epithelial cells and fibroblasts). Dendritic cells are involved in the initiation of innate immunity in kidney ischemia-reperfusion injury and induce injury either through direct cellular contact or through the release of soluble mediators. The early immune response consists of activation of dendritic cells and macrophages that produce cytokines and chemokines leading to influx of leukocytes (43, 47). Macrophage and dendritic cell subpopulations (3, 44, 48, 49), neutrophils (50), and lymphocytes, particularly CD4⁺ T cells and B cells, are thought to contribute to kidney ischemia-reperfusion injury (51-53). Dendritic cells activate CD4⁺ natural killer T cells and promote inflammation (50, 54). Following establishment of injury, regulatory T cells and macrophage subsets suppress the kidney ischemia-reperfusion injury or initiate repair processes (48, 55, 56).

The complement system is central in the pathogenesis of several types of AKI. Three separate initiation pathways (classical, alternative and lectin) converge on a common terminal amplification step involving C3 and C5 and resulting in the formation of the membrane attack complex. In addition to formation of the membrane attack complex, the subunits of C3 (e.g. C3a) and C5 (e.g. C5a) have pro-inflammatory actions on their own. These subunits bind to G protein-coupled receptors to induce cytokine and chemokine production and stimulate both innate and adaptive immune responses during kidney injury (57-59). Inhibition of the membrane attack complex formation does not appear to prevent injury to tubular epithelial cells induced by cisplatin (60), whereas C5a deficiency significantly inhibits neutrophil infiltration, tubular epithelial cell apoptosis, and loss of kidney function after cisplatin treatment in mice. Increased urinary levels of a C5 subunit in humans after cisplatin administration provide clinical evidence suggesting a role of complement activation in AKI (61).

In animal models of kidney ischemia-reperfusion injury and in kidney samples from patients with acute tubular necrosis, evidence for activation of the alternative pathway of complement has been demonstrated (62, 63). Mice deficient in factor B, a critical component of the alternative pathway, suffer less severe kidney injury after ischemia reperfusion (63). In contrast, deficiency of C4, which is a component of both the classical and lectin complement initiation pathways, was not protective against kidney ischemia-

reperfusion injury (64). Normally the epithelial cells lining the proximal tubules of the kidney express the complement inhibitor Crry (complement receptor 1-related protein y) preferentially on the basolateral membrane (65). During reperfusion, Crry is redistributed away from the basolateral surface of the cell permitting deposition of C3 on the tubular epithelium. Accordingly, mice deficient in Crry are more susceptible to kidney ischemia-reperfusion injury. The C3a subunit is required for the production of the pro-inflammatory chemokines macrophage inflammatory factor 2 (MIP-2) and keratinocyte-derived chemokine (KC) by the renal tubular epithelium after renal ischemia (59). These chemokines attract neutrophils and macrophages to the injured kidney. In summary, ischemic and nephrotoxic AKI involve complement activation.

Peroxisome Proliferator-Activated Receptors

The peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors that influence gene expression. The PPARs regulate diverse cellular functions including lipid metabolism, cellular differentiation, inflammatory responses, and cell survival (66). There are 3 subgroups of PPARs named PPAR- α , PPAR- γ and PPAR- β/δ all of which appear to play protective roles in animal models of AKI (67-71). For example, mice deficient in PPAR- β/δ are more susceptible to ischemia-reperfusion-induced decreased kidney function and tubular necrosis (71).

In both ischemic and cisplatin-induced kidney injury models, PPAR- α -deficient mice suffer worse injury (67, 68). The role of PPAR- α in AKI has been carefully studied over the last decade and several mechanisms of protection have been elucidated. Insults, such as ischemia and cisplatin, decrease the expression and activity of multiple enzymes involved in cellular metabolism and homeostasis (i.e. fatty acid oxidation and glucose metabolism). PPAR- α ligand administration or proximal tubule-specific over-expression of PPAR- α is sufficient to prevent loss of these enzymes and suppress kidney injury (67, 68, 72). Proximal tubule-specific PPAR- α induction was also associated with decreased accumulation of the lipid peroxidation product 4-hydroxy-2-hexenal, which is known to induce mitochondrial dysfunction and apoptosis, and promote inflammation (72). In addition, the cisplatin-induced increase in endonuclease G expression and activity, which leads to tubular epithelial cell death, was potently inhibited by administration of a PPAR- α ligand in PPAR- α ^{+/+} mice, but not PPAR- α ^{-/-} mice (68). Finally, PPAR- α inhibits pro-inflammatory cytokine, chemokine, and chemokine receptor expression as well as adhesion molecule upregulation induced by cisplatin, adding another mechanism of protection(73).

Proximal tubule epithelial cell death, excessive inflammation, and impairment of kidney cellular metabolism and homeostasis are 3 well-studied pathogenic mechanisms which promote AKI. The elucidation of these mechanisms has formed the basis for the recent advances in potential therapies for AKI presented below.

RECENT ADVANCES

Targeting Cell Death Pathways

Demonstration that apoptotic cell death significantly contributes to loss of kidney function has opened new opportunities to prevent and treat AKI. Tubular cell apoptosis and loss of kidney function were prevented *in vivo* by using a pharmacological inhibitor of mitochondrial fragmentation(19). Use of a proteasome inhibitor blocked the degradation of Mcl-1 and therefore mitochondrial release of AIF, attenuating decreased kidney function induced by cisplatin (26). In animal models, use of the p53 inhibitor pifithrin- α decreases apoptosis and loss of kidney function induced by cisplatin(74), ischemia (29), and aristolochic acid(32). Furthermore, siRNA (small interfering RNA) directed against p53 for gene silencing is rapidly and preferentially taken up by tubular epithelial cells after

intravenous injection, and suppresses apoptosis and kidney dysfunction induced by cisplatin and ischemia-reperfusion injury in rats (31). Pancaspase inhibitors and p53 inhibitors are in currently in clinical trials for liver transplant (ClinicalTrials.gov identifier NCT00080236) and acute kidney injury (NCT00683553), respectively

Suppression of the Immune Response to Kidney Injury

Understanding the immune basis of kidney ischemia-reperfusion injury has spawned many studies aimed at modulating these pathways through small molecules that specifically target these cells. Studies indicate that adenosine A2a receptor (A2aR) agonists target CD4⁺ T cells and other bone marrow-derived cells to inhibit kidney ischemia-reperfusion injury (53, 75). Activation of sphingosine-1-phosphate receptor 1 (S1PR1) on immune cells and proximal tubular cells, using agents such as FTY720, protect mice from kidney ischemia-reperfusion injury (76, 77). A selective A2aR agonist is approved for radionuclide myocardial perfusion imaging, and FTY720 is approved for the treatment of relapsing multiple sclerosis. Targeting the complement system using a C5a receptor antagonist (58) and specific C5a siRNA administration (78) prior to ischemia suppresses ischemia-reperfusion injury in mice. Of note, the C5a receptor antagonist, PMX53, was recently used in rheumatoid arthritis patients without significant adverse effects (79). Novel cell-based therapies demonstrate the potential to program and induce phenotypic changes of macrophages to initiate repair processes in mice (48, 80). Another cell-based therapy involves adoptive transfer of regulatory T cells, which are very effective at reducing kidney inflammation and injury when administered prior to various kidney insults (81, 82). Furthermore, adoptive transfer of regulatory T cells as late as 24 hr after ischemia significantly accelerates recovery from ischemia-reperfusion injury in mice (55). The safety and feasibility of *ex vivo* expansion and adoptive transfer of regulatory T cells to humans has been demonstrated in a recently published clinical trial (83).

PPAR Activation

In contrast to the use of inhibitors of cell death or inflammation, promoting PPAR activity may lessen the severity of AKI by promoting cellular metabolism and homeostasis, and inhibiting inflammation. Administration of the PPAR- β/δ agonist L165041 prior to ischemia prevented loss of kidney function and kidney cell apoptosis *in vivo*. It also blocked oxidant-induced apoptosis of cultured human proximal tubular cells *in vitro* (71). Members of one class of PPAR- γ ligands, the thiazolidinediones, have also shown benefits when administered orally prior to kidney ischemia (70) and when given intravenous during ischemia and shortly after initiating reperfusion (69). Troglitazone suppressed caspase 3 cleavage and DNA fragmentation in the kidney lessening the severity of both ischemia-induced cell death and kidney dysfunction (70). Rosiglitazone and ciglitazone inhibited the expression of intercellular adhesion molecule 1 (ICAM-1) and accumulation of neutrophils in the post-ischemic kidney providing significant protection of kidney function and histology (69). These findings are encouraging given that several thiazolidinediones are already approved by the US FDA for use in diabetes management. Fibrates, which are used clinically for hyperlipidemia, are stimulating ligands for PPAR- α and have been used successfully in some of the preclinical models of AKI discussed above. In summary, activating PPARs in animal models of AKI is protective, and several PPAR activators are currently approved for use in humans.

Bone Marrow Multipotent Stromal Cells

Another cell-based therapy involves adoptive transfer of bone marrow-derived multipotent stromal cells (MSCs; also referred to as mesenchymal stem cells). These cells reduce injury in pre-clinical cisplatin (84), ischemia-reperfusion injury (85, 86), and glycerol-induced kidney injury models (87). The mechanisms of protection include reduction of tubular cell

apoptosis and promotion of tubular cell proliferation. Currently, a clinical trial involving adoptive transfer of bone marrow MSCs to patients at high risk of AKI after undergoing cardiac surgery is underway (NCT00733876).

Thrombomodulin and Activated protein C

Thrombomodulin and endothelial protein C receptors (EPCRs) expressed on endothelial cells activate protein C, and promote anticoagulation (88). Soluble thrombomodulin, independent of its ability to generate activated protein C (APC), reduced kidney ischemia-reperfusion injury by improving microvascular flow dynamics, reducing endothelial leukocyte adhesion, and minimizing endothelial permeability (89). APC has also been shown to have direct cellular anti-inflammatory, anti-apoptotic activities, and promote stability of the endothelial barrier function via EPCRs (88, 90-95). Current effort has been devoted to generating APC variants that have cytoprotective properties and minimal anticoagulant properties (96) (97). Thus genetically engineered APC variants and thrombomodulin might yield specific agents that take advantage of selective anticoagulant and cytoprotective properties in future clinical studies of AKI in sepsis or in critically ill patients.

Recombinant Erythropoietin

Erythropoietin receptors may serve as a critical target in cell survival. Binding of erythropoietin to its receptor on target tissue leads to initiation of complex intracellular signaling pathways (98-100), reduction of kidney injury by reducing tubular necrosis and apoptosis (101-103), and activation of the phosphoinositide 3 kinase (PI3K)/Akt pathway (104) or heat shock protein 70 (Hsp70) (105). Recombinant erythropoietin has also been demonstrated to participate in the repair process of kidney injury. In a cisplatin model of acute kidney injury, recombinant erythropoietin enhanced tubular proliferation (106). To the extent that endothelial progenitor cells may participate in tissue repair, recombinant erythropoietin has been shown to induce these cells from bone marrow to mobilize and proliferate (107, 108). The tissue-protective properties of recombinant erythropoietin appear to require a physical association between an 11-amino acid peptide composed of adjacent amino acids of helix B and a peptide non erythropoietic fragment containing amino acid residues 58-82 (109, 110). Thus recombinant erythropoietin or non-erythropoietic peptide fragments are agents that have promise in the treatment of AKI despite an early negative study (111).

Heme Oxygenase, Carbon Monoxide and Bilirubin

In 1992, Nath *et al.* found that heme oxygenase (HO) induction was important in limiting the extent of rhabdomyolysis-induced AKI (112). HO is the rate-limiting enzyme in the degradation of heme, leading to the production of biliverdin carbon monoxide and biliverdin (113). It is thought that the products of HO metabolism, carbon monoxide, bilirubin, and biliverdin, exert cytoprotective effects (112, 114). In kidney ischemia-reperfusion injury, bilirubin has also been shown to reduce kidney injury (115). In heart allografts, the combination of biliverdin and carbon monoxide yielded synergistic effects on graft survival (116).

Carbon monoxide induces HIF-1 α , which has a number of target genes including HO (117) that could lead to local tissue protection. Recently CD4⁺CD25⁺ T regulatory cells activated by antigen presenting cells reduce the extent of injury in ischemia-reperfusion injury (56). George *et al.* demonstrated that the suppressive effect of T regulatory cells is dependent on activation by antigen presenting cells that express HO (118). In the absence of HO, suppressive effects of regulatory T cells were abolished. These findings point to the

important role that HO-1 activity in antigen-presenting cells play in mediating T regulatory cell suppressive activity.

As systemic delivery of carbon monoxide can be toxic, the potential for tissue specific release of carbon monoxide has drawn attention recently. Compounds that bind carbon monoxide and release at the tissue may circumvent toxicity and reduce tissue injury. Carbon monoxide donor compounds have been developed and are protective when administered before ischemia (119) or lipopolysaccharide administration (120). Thus carbon monoxide donors hold great promise as they permit cytoprotection in the absence of systemic toxicity. Alternatively, low therapeutic doses of carbon monoxide may circumvent toxicity and yield cytoprotection.

Alkaline Phosphatase

Alkaline phosphatase (AP) is expressed abundantly in kidney tissue and in other cells and organs (121) (122). In experimental studies, AP is inactivated by reactive oxygen species and peroxynitrite. When administered AP reduces inflammation and mortality in sepsis (123-127) forming the basis for a multicenter randomized placebo control trial in 36 critically ill patients followed for nearly 24 hrs(128). The AP group was associated with reduced excretion of a proximal tubule biomarker, glutathione S-transferase A1-1, NO excretion, and improved kidney function (128).

SUMMARY

In returning to the case vignette, there are several points that are worth highlighting. This patient had multiple factors that contributed to AKI including 1) hypotension leading to ischemia-reperfusion injury, 2) bacterial sepsis, 3) nephrotoxic drugs including acyclovir, cyclophosphamide, and aminoglycoside, and 4) hemoglobin. The cumulative effect of these factors is the full expression of injury. Ischemia-reperfusion may lead to endothelial and epithelial cellular cytotoxicity and immune mediated injury. Sepsis may contribute to activation of Toll-like receptors leading to pathogen-associated molecular pattern (PAMP) and damage-associated molecular pattern (DAMP) mediated activation of the immune response and AKI. Acyclovir may lead to crystal induced AKI, and aminoglycosides and hemoglobin are direct cellular toxins. Thus the multifactorial nature of AKI in this case illustrates the importance of understanding the complex pathogenesis in order to implement rationale therapies that may target more than one pathway through combination drug therapy (Figure 3). Although rationale in design, combination therapy adds additional complexity in the analysis of safety and efficacy needed for drug approval by the FDA.

In conclusion, clinical trials so far have shown that targeting a single mechanism may not be sufficient to provide significant benefit. There are a number of reasons that have led to failed clinical trials despite successful animal studies. The patient population studied is heterogeneous with a number of co-morbidities, and they are often older, critically ill, and have concurrent multi-organ failure. There has been a lack of a standardized definition of AKI and accepted endpoints for clinical trials. Lastly, failed clinical trials have been hampered by low statistical power, poorly timed administration of the drug, and adverse effects of the drug. Thus, in order to make advances in reducing the morbidity and mortality, a concerted effort is needed among scientists to define the pathogenesis of AKI, academic and industry clinical trialists to design studies, and research advisors at the National Institutes of Health (NIH) and FDA to translate these findings to human AKI. Major advances were made following the recent AKI Clinical Trials Workshop (December 2-3, 2010; Natcher Conference Center, NIH Campus, Bethesda, MD) that brought together academic investigators, industry partners, and members from NIH and the FDA. The result of this meeting will open communication between different sectors to advance the

cooperation necessary for the genesis of clinical trials to stem the morbidity and mortality of AKI.

Box 1

New Agents for Treatment of AKI

- **Anti-apoptosis/necrosis agents**
 - ◇ Caspase inhibitors
 - ◇ Minocycline
 - ◇ Pifithrin- α (p53 inhibitor)
 - ◇ PARP inhibitor
- **Anti-sepsis**
 - ◇ Activated protein C
- **Growth factors**
 - ◇ Recombinant erythropoietin
- **Vasodilators**
 - ◇ Carbon Monoxide release compounds
 - ◇ Bilirubin
- **Anti-inflammatory drugs**
 - ◇ Sphingosine-1-phosphate analogs
 - ◇ Adenosine 2A agonists
 - ◇ Adenosine analogs
 - ◇ iNOS inhibitors
 - ◇ Fibrates
 - ◇ Thiazolidinediones
 - ◇ Alkaline phosphatase
- **Cell-based therapies**
 - ◇ M2 macrophages
 - ◇ Regulatory T cells
 - ◇ Bone marrow multipotent stromal cells

Abbreviations: ADP, adenosine diphosphate; AKI, acute kidney injury; iNOS, inducible nitric oxide synthase; PARP, poly(ADP-ribose) polymerase

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Figure 1.
Plasma and Urine sample. Collected samples demonstrated hemolysis and free hemoglobin in the plasma, and the urine specimen tested positive for blood.

Figure 2.

Immune mechanisms in kidney ischemia-reperfusion injury (IRI). The insult activates a number of targets including leukocytes, endothelial cells, and tubular epithelial cells leading to a “sterile” inflammatory response. This process is necessary to initiate repair process but overzealous activation leads to tissue injury. Invariant natural killer T (NKT) cells, neutrophils (PMN), and macrophages (MØ), which originate in the bone marrow, collect in the kidney where they are activated and release pro-inflammatory cytokines. IRI has detrimental effects on endothelial cells, resulting in greater vascular permeability, expression of adhesion molecules, and leukocyte migration into the kidney. Renal dendritic cells (DCs) produce cytokines and chemokines that respond to damage-associated molecular pattern (DAMP) signals, activate NKT cells, and produce cytokines and chemokines. Tubular epithelial cells show enhanced complement deposition and upregulate the expression of Toll-like receptors (TLRs), both of which mediate chemokine and cytokine production in the injured kidney. As a direct or indirect consequence of changes in each cell type, other cells drive inflammation after kidney IRI. These interactions between kidney and bone marrow derived cells, and innate and adaptive immunity, reflect the complexities in acute kidney injury-associated inflammation. This figure is based on a model presented in Kinsey et al(3).

Figure 3.

Sites of action for selected therapeutic agents with potential use in acute kidney injury (AKI). Abbreviations: PPAR, peroxisome proliferator-activated receptor; A2aR, adenosine A2a receptor; S1PR1, sphingosine-1-phosphate receptor 1; Tregs, regulatory T cells; MSCs, multipotent stromal cells; CO, carbon monoxide; APC, activated protein C; EPO, erythropoietin.