Bridging translation for acute kidney injury with better preclinical modeling of human disease

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Skrypnyk NI, Siskind LJ, Faubel S, de Caestecker MP. Bridging translation for acute kidney injury with better preclinical modeling of human disease. Am J Physiol Renal Physiol 310: F972–F984, 2016. First published March 9, 2016; doi:10.1152/ajprenal.00552.2015.—The current lack of effective therapeutics for patients with acute kidney injury (AKI) represents an important and unmet medical need. Given the importance of the clinical problem, it is time for us to take a few steps back and reexamine current practices. The focus of this review is to explore the extent to which failure of therapeutic translation from animal studies to human studies stems from deficiencies in the preclinical models of AKI. We will evaluate whether the preclinical models of AKI that are commonly used recapitulate the known pathophysiology of AKI that are being modeled in humans, focusing on four common scenarios that are studied in clinical therapeutic intervention trials: cardiac surgery-induced AKI; contrast-induced AKI; cisplatin-induced AKI; and sepsis associated AKI. Based on our observations, we have identified a number of common limitations in current preclinical modeling of AKI that could be addressed. In the long term, we suggest that progress in developing better preclinical models of AKI will depend on developing a better understanding of human AKI. To this end, we suggest that there is a need to develop greater in-depth molecular analyses of kidney biopsy tissues coupled with improved clinical and molecular classification of patients with AKI.

acute kidney injury; animal models; human disease; preclinical research; drug discovery

ACUTE KIDNEY INJURY (AKI) is characterized by an abrupt deterioration in kidney function that disrupts metabolic, fluid and electrolyte homeostasis over a period of hours or days. An analysis of 305 AKI cohort studies found that AKI occurred in 21% of adults hospitalized in developed countries with an acute illness (153). The risk of AKI is increased in the elderly, in patients with preexisting chronic kidney disease (CKD), and in patients with diabetes mellitus (130). An episode of AKI is also a strong predictor of mortality: in patients with severe AKI requiring dialysis mortality approaches 60% in some series (130).

US and European guidelines for the management of patients with AKI outline a series of diagnostic, renal support strategies and approaches to reduce further damage to the kidney (8a, 70). However, other than optimization of hemodynamics and avoidance of nephrotoxins, no therapeutic interventions have been shown to improve clinical outcomes in patients with AKI (31, 104, 117). Given the high mortality and morbidity associated with AKI, there is an important unmet medical need to develop effective therapies for these patients. This failure cannot be attributed to a lack of preclinical research or from a failure to conduct clinical trials (72). Indeed, numerous potential therapies for AKI have been identified from preclinical research (17, 95, 127, 154). Unfortunately, most of the phase 2 proof-of-concept trials in AKI have yielded conflicting results, and none of the therapeutic interventions tested in definitive phase 3 trials have shown improvements in clinically meaningful end points (25, 103, 112, 173).

This translational gap between preclinical efficacy studies in animal models and clinical trials in patients with AKI may be occurring for a number of different reasons. These include 1) deficiencies in clinical study design and power (68, 118, 173); 2) failure to identify and distinguish between patients with different pathophysiological subtypes of AKI (34); 3) lack of definitive AKI biomarkers that are predictive of clinically meaningful outcomes and/or responses to therapeutic interventions (41); and 4) analysis of surrogate measures that do not predict improvements in long-term clinically meaningful renal outcomes after AKI (25). However, there is also concern that the animal models may not be predictive of therapeutic responses in humans with AKI (39, 45, 57, 114, 137). In this review, we discuss some of the reasons current preclinical models of AKI may not be translated into clinical practice and will outline strategies that could enhance the translatability of preclinical AKI research.
Preclinical Models of Human AKI

There has been an effort to develop preclinical models that more closely mimic injury in patients with AKI (113). Ideally, preclinical AKI models should be tailored to model specific clinical scenarios that are being planned for future clinical intervention trials. However, in practice this is rarely done. To illustrate this, we have surveyed the preclinical studies that have been used to support completed phase 2 and phase 3 AKI studies registered with www.clinicaltrials.gov (Tables 1 and 2). All clinical trials for drugs that might be marketed in the United States have been registered on this website since 2007. We have focused on clinical studies involving therapeutic interventions and have only included those studies that were annotated as “completed” in the database. Therefore, while our search does not include all of the clinical trials in AKI, it provides a snapshot of clinical studies that have been performed along with the preclinical data that have been used to support these therapeutic intervention studies. Studies meeting these criteria are restricted to four clinical scenarios: cardiac surgery-associated AKI (CSA-AKI), contrast-induced AKI (CI-AKI), cisplatin-induced AKI (CP-AKI), and sepsis-associated AKI (SA-AKI).

CSA-AKI

Cardiac surgery has the highest postoperative risk of AKI compared with other major surgical procedures (55). Up to 30% of patients undergoing cardiac surgery develop AKI, and ~1% of these patients require dialysis (134). Despite declining numbers of cardiac surgical procedures, those that are being performed are often more complex procedures that take longer to perform and are associated with an increased incidence of procedure-associated AKI (83, 161). CSA-AKI is an independent risk factor for mortality after cardiac surgery (126), and patients with CSA-AKI are at increased risk of CKD, particularly those patients with AKI requiring dialysis (86, 175).

Common risk factors for CSA-AKI include preexisting CKD, old age, diabetes, and the female gender (134). Other risk factors include hemodynamic instability, cardiac failure, the type and length of the procedure, and use of cardiopulmonary bypass (CPB). In addition, patients who develop contrast-induced AKI following coronary angiography, which is often performed before surgery, are at increased risk of a second episode of AKI after cardiac surgery (51). A large number of therapeutic intervention studies have been performed, mostly prevention studies in which test drugs were administered before or at the time of cardiac surgery, but none have shown consistent beneficial effects on CSA-AKI incidence or outcomes (120, 179). In part, this can be attributed to weaknesses in study design since many of these studies only evaluated short-term effects on serum creatinine levels and were underpowered to detect clinically significant end points such as death, dialysis, and CKD (145).

Table 1. Clinical trials for AKI

<table>
<thead>
<tr>
<th>Drug</th>
<th>Indication</th>
<th>Risk Factors</th>
<th>Timing of Therapy</th>
<th>Result</th>
<th>Reference or Study Identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-acetylcysteine + fenoldopam</td>
<td>CSA-AKI</td>
<td>CKD</td>
<td>Prevention</td>
<td>Positive</td>
<td>15</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>CSA-AKI</td>
<td>CKD</td>
<td>Prevention</td>
<td>No effect</td>
<td>101</td>
</tr>
<tr>
<td>Erythropoietin (or erythropoietin analogs)</td>
<td>CSA-AKI</td>
<td>CKD</td>
<td>Prevention</td>
<td>Positive</td>
<td>159</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None</td>
<td>Prevention</td>
<td>Positive</td>
<td>111</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Includes</td>
<td>Postoperative</td>
<td>No effect</td>
<td>39</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td></td>
<td>CKD</td>
<td>Prevention</td>
<td>No effect</td>
<td>47</td>
</tr>
<tr>
<td>Rasburicase</td>
<td></td>
<td>CKD</td>
<td>Prevention</td>
<td>No effect</td>
<td>35</td>
</tr>
<tr>
<td>Neseritide (brain natriuretic peptide)</td>
<td></td>
<td>CKD</td>
<td>Prevention</td>
<td>Positive</td>
<td>164</td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td>CKD</td>
<td>Prevention + 12 h</td>
<td>Worse vs. NaCl</td>
<td>76</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td></td>
<td>CKD/DM</td>
<td>Prevention</td>
<td>No effect</td>
<td>7</td>
</tr>
<tr>
<td>Delfiprone (iron chelator)</td>
<td></td>
<td>CKD/DM</td>
<td>Prevention</td>
<td>Unknown</td>
<td>NCT01146925</td>
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<tr>
<td>Vitamin E + N-acetylcysteine</td>
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<td>CKD/DM</td>
<td>Prevention</td>
<td>Unknown</td>
<td>NCT02070679</td>
</tr>
<tr>
<td>Vitamin E</td>
<td></td>
<td>CKD/DM</td>
<td>Prevention</td>
<td>Positive</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CKD</td>
<td>Prevention</td>
<td>Unknown</td>
<td>NCT01562925</td>
</tr>
<tr>
<td>Wine/beer</td>
<td></td>
<td>CKD/DM</td>
<td>Prevention</td>
<td>Unknown</td>
<td>142</td>
</tr>
<tr>
<td>Silymarin</td>
<td>CP-AKI</td>
<td>CKD</td>
<td>Prevention</td>
<td>Unknown</td>
<td>NCT00676234 Part reported in Ref. 39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None</td>
<td>Presence of SIRS</td>
<td>No effect (toxicity noted)</td>
<td>138</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>SA-AKI</td>
<td>CKD</td>
<td>Prevention</td>
<td>Unknown</td>
<td>NCT00676234 Part reported in Ref. 39</td>
</tr>
<tr>
<td>Erythropoietin</td>
<td></td>
<td>CKD</td>
<td>Prevention</td>
<td>Unknown</td>
<td>NCT00676234 Part reported in Ref. 39</td>
</tr>
<tr>
<td>Hydroxyethyl starch (HES)</td>
<td>SA-AKI</td>
<td>None</td>
<td>For volume resuscitation</td>
<td>Worse</td>
<td>124</td>
</tr>
<tr>
<td>Insulin</td>
<td>SA-AKI</td>
<td>None</td>
<td>For volume resuscitation</td>
<td>No effect</td>
<td>11</td>
</tr>
<tr>
<td>Insulin + HES</td>
<td>SA-AKI</td>
<td>None</td>
<td>For volume resuscitation</td>
<td>Worse</td>
<td>28</td>
</tr>
</tbody>
</table>

AKI, acute kidney injury. See text for additional definitions. Clinical studies were identified from www.clinicaltrials.gov using search terms “acute kidney injury” and “phase 2” or “phase 3” studies. Only completed studies that evaluated drug interventions and in which efficacy was evaluated with renal function, dialysis, or death as primary end points appear. Renal transplantation and cell-based therapies were excluded. Clinical study identification number from the www.clinicaltrials.gov site is provided when published references are not available. Timing of therapy refers to timing in relation to the initiating event. Prevention studies include interventions that were delivered at any time before or at the same time as the initiating event.
Pathophysiology of CSA-AKI. The pathogenesis of CSA-AKI is almost certainly multifactorial. A variety of mechanisms have been implicated. These include 1) reduced renal perfusion; 2) decreased autoregulation of renal blood flow; 3) hemodilution, resulting in reduced oxygen-carrying capacity of blood perfusing the kidney; 4) systemic inflammation caused by passage of blood through the bypass circuits and from leakage of bacterial endotoxins from the intestine; 5) renal ischemia or infarctions caused by macroscopic and microscopic renal embolization; 6) shear stress-induced intravascular hemolysis giving rise to pathogenic oxidative stress from excess free hemoglobin; 7) perioperative and postoperative hemodynamic instability; and 8) drug toxicities (56, 93, 134). Histological analyses of the kidneys from patients with CSA-AKI are limited to early autopsy series and indicate that while a proportion of patients have "normal" renal histology (177). While this study was conducted more than 40 years ago and many of the surgical procedures and interventions associated with CSA-AKI have changed substantially, these data suggest that there maybe profound effects on cellular and physiological functions in the kidney without inducing histological evidence of cellular injury in many patients with CSA-AKI.

Clinical trials and preclinical models for CSA-AKI. All of the clinical trials in our review of patients with CSA-AKI were trials to prevent AKI rather than to treat patients with established AKI (Table 1). Cardiac surgery provides a convenient model for studying the effects of interventions developed to prevent AKI, since the timing of injury is usually well defined and risk factors for AKI are well known. In addition, patients are admitted to the hospital before the procedure and kept in the hospital for a number of days after the procedure, facilitating follow-up and selection of patients at risk for developing CSA-AKI. Of the clinical studies that we identified, five of

<table>
<thead>
<tr>
<th>Drug</th>
<th>Model</th>
<th>Species</th>
<th>Timing of Therapy</th>
<th>Dose-Response</th>
<th>Target Analysis</th>
<th>Results</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenoldopam</td>
<td>IR-AKI</td>
<td>Rats</td>
<td>Prevention</td>
<td>No</td>
<td>No</td>
<td>Positive</td>
<td>9</td>
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<tr>
<td></td>
<td>Community-acquired AKI</td>
<td>Cats+dogs</td>
<td>Established</td>
<td>No</td>
<td>No</td>
<td>Negative</td>
<td>109</td>
</tr>
<tr>
<td>N-acetylcysteine</td>
<td>IR-AKI</td>
<td>Rats</td>
<td>Prevention</td>
<td>Yes</td>
<td>No</td>
<td>Positive</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>CPB-AKI</td>
<td>Rats</td>
<td>Prevention</td>
<td>Yes</td>
<td>Yes</td>
<td>Positive</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>CI-AKI</td>
<td>Rats</td>
<td>Prevention</td>
<td>No</td>
<td>No</td>
<td>Positive</td>
<td>178</td>
</tr>
<tr>
<td></td>
<td>CLP-AKI</td>
<td>Rats</td>
<td>Prevention</td>
<td>No</td>
<td>Yes</td>
<td>Positive</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Glycercol</td>
<td>Rats</td>
<td>Prevention</td>
<td>No</td>
<td>No</td>
<td>Positive</td>
<td>74</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>IR-AKI</td>
<td>Rats</td>
<td>Prevention</td>
<td>No</td>
<td>Yes</td>
<td>No effect</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>Mercucr chloride</td>
<td>Rats</td>
<td>Prevention</td>
<td>No</td>
<td>No</td>
<td>Worse vs. NaCl</td>
<td>19</td>
</tr>
<tr>
<td>Erythropoietin (or erythropoietin analogs)</td>
<td>IR-AKI</td>
<td>Rats</td>
<td>Prevention</td>
<td>No</td>
<td>No</td>
<td>Positive</td>
<td>166</td>
</tr>
<tr>
<td></td>
<td>Glycerol</td>
<td>Rats</td>
<td>Prevention</td>
<td>No</td>
<td>No</td>
<td>Positive</td>
<td>146</td>
</tr>
<tr>
<td></td>
<td>IR-AKI</td>
<td>Macaques</td>
<td>Prevention</td>
<td>No</td>
<td>No</td>
<td>Positive</td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>CP-AKI</td>
<td>Rats</td>
<td>Prevention</td>
<td>No</td>
<td>No</td>
<td>Positive</td>
<td>67</td>
</tr>
<tr>
<td>Debferoxamine (iron chelator)</td>
<td>Glycerol</td>
<td>Rats</td>
<td>Prevention</td>
<td>Yes</td>
<td>Yes</td>
<td>Positive</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>UOO</td>
<td>Mice</td>
<td>Prevention</td>
<td>No</td>
<td>Yes</td>
<td>Positive</td>
<td>131</td>
</tr>
<tr>
<td>Deferoxamine + N-acetylcysteine</td>
<td>Gentamicin</td>
<td>Rats</td>
<td>Prevention</td>
<td>No</td>
<td>Yes</td>
<td>Positive</td>
<td>125</td>
</tr>
<tr>
<td>Vitamin E + N-acetyl cysteine</td>
<td>Gentamicin</td>
<td>Rats</td>
<td>Prevention +8 days post</td>
<td>Yes</td>
<td>Yes</td>
<td>Positive</td>
<td>20</td>
</tr>
<tr>
<td>Vitamin E + pentoxifylin</td>
<td>Glycerol</td>
<td>Rats</td>
<td>Prevention</td>
<td>No</td>
<td>No</td>
<td>No effect</td>
<td>6</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Mercucr chloride</td>
<td>Rats</td>
<td>Prevention +2 days post</td>
<td>No</td>
<td>Yes</td>
<td>Positive</td>
<td>5</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>IR-AKI</td>
<td>Rats</td>
<td>Prevention</td>
<td>No</td>
<td>No</td>
<td>Positive</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>Hypovolemic Shock</td>
<td>Rats</td>
<td>Resuscitate</td>
<td>No</td>
<td>Yes</td>
<td>No effect</td>
<td>169</td>
</tr>
<tr>
<td></td>
<td>IR-AKI</td>
<td>Rats</td>
<td>Prevention</td>
<td>No</td>
<td>Yes</td>
<td>Positive</td>
<td>21, 73</td>
</tr>
<tr>
<td></td>
<td>Glycerol</td>
<td>Rats</td>
<td>Prevention</td>
<td>Yes</td>
<td>Yes</td>
<td>Positive</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Lipopolysaccharide</td>
<td>Mice</td>
<td>Prevention</td>
<td>No</td>
<td>No</td>
<td>No effect</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>CLP-AKI</td>
<td>Mice</td>
<td>Prevention</td>
<td>No</td>
<td>Yes</td>
<td>Positive</td>
<td>61</td>
</tr>
<tr>
<td>Silymarin</td>
<td>CP-AKI</td>
<td>Rats</td>
<td>Prevention +2 days post</td>
<td>No</td>
<td>No</td>
<td>Positive</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>HES</td>
<td>Lipopolysaccharide</td>
<td>Rats 1 h post</td>
<td>No</td>
<td>No</td>
<td>No effect</td>
<td>167</td>
</tr>
</tbody>
</table>

UO, unilateral ureteral obstruction. See the text for additional definitions. Preclinical studies were identified from PubMed and Google searches using generic, chemical, and/or brand names of drugs and “acute kidney injury” or “kidney injury” search terms. If multiple examples of the same model were found, only 1–2 references were included. Timing of therapy refers to timing in relation to the initiating event. Prevention refers to interventions that were delivered before or at the same time as the initiating event. Target analysis refers to analysis of known molecular targets of drug action.
Eight were performed in patients with preexisting CKD, and all of the studies evaluated short-term effects of the interventions on postoperative changes in serum creatinine. One study evaluated long-term effects on all-cause mortality but only as a secondary end point (111).

Of the animal studies used to support the use of the therapeutic interventions for patients with CSA-AKI summarized in Table 1 [fenoldopam, N-acetyl-cysteine (NAC), sodium bicarbonate, erythropoietin analogs, rasburicase, nesiritide (BNP), and insulin], 8 of 23 of the studies used ischemia-reperfusion models of AKI (IR-AKI) and the majority evaluated the effects of treatment regimens designed to prevent rather than treat established AKI (Table 2). The majority of IR-AKI studies were performed in rats, but one IR-AKI study was performed in macaques (66). The rationale for using IR-AKI models is based on the observation that there is reduced renal blood flow during CPB and that this, along with reduced oxygen-carrying capacity of the diluted blood, may result in critical ischemia to metabolically active cells in the kidney. Other AKI models that were used to support use of these therapeutic interventions in patients with CSA-AKI included the cecal ligation and perforation (CLP) and endotoxin (LPS) models of sepsis in rats and dogs, respectively, as well as glycerol-induced rhabdomyolysis in rats. Each of these models mimics some but not all of the pathophysiological features of CSA-AKI. For example, there is activation of systemic inflammatory responses in the LPS, CLP, glycerol-induced AKI, and IR-AKI models, each of which may mimic some aspects of the systemic inflammatory response seen in patients after CPB (56, 60, 87, 134). Some drugs were shown to be effective in multiple models of AKI. Examples of this include the use erythropoietin analogs and NAC, both of which are effective in IR-AKI, CLP, and glycerol models of AKI. In addition, both NAC and erythropoietin analogs were evaluated in a rat model of CPB (168, 180). However, despite the demonstrable efficacy of these therapeutic interventions across multiple preclinical models, mixed results of clinical trials using the same interventions suggest that efficacy in multiple models, including the rat model of CPB, is not a guarantee that preclinical AKI studies will translate into effective therapeutics for patients with CSA-AKI. In part, failure of these preclinical therapeutic efficacy studies to translate into clinical practice may be because none of the preclinical studies incorporated known CSA-AKI risk factors, notably old age, CKD, and diabetes mellitus. These could potentially influence therapeutic responses to therapy. Other models have been developed that may better mimic clinical scenarios, including pig models of CPB (91, 105). However, these pig models are only available in a small number of specialized laboratories, and none of the published data that we were able to identify using pig models of CPB have extended beyond early time points after the surgical intervention (52, 53, 91, 96, 108, 121–123, 148, 150). Furthermore, it is unknown whether these large-animal models will better recapitulate the pathophysiology of human disease in which comorbidities contribute to AKI susceptibility. For example, a recent study suggests that obesity, which is one of the risk factors for CSA-AKI in patients (110), may actually protect against AKI after CPB in pigs (150).

CI-AKI

Iodinated contrast agents are used to optimize imaging for different X-ray imaging techniques. CI-AKI may have a varied course but is typically characterized by a rise in serum creatinine 2–3 days after contrast administration (99). While only a small number of patients develop dialysis-dependent AKI, a significant proportion of these patients do not recover baseline kidney function (54, 99). Moreover, patients who develop CI-AKI are at increased risk of serious cardiac events even if their serum creatinine values return to baseline (54, 99). Risk factors for CI-AKI include old age, female sex, CKD, and diabetes mellitus (16). In addition, the site of contrast administration can play a role. For example, AKI is more common after percutaneous coronary interventions in which contrast is given via intra-arterial injection than after intravenous contrast injections commonly used for CT imaging studies (10, 100, 107). The risk of CI-AKI is also affected by the volume of contrast agent given as well as the presence of hemodynamic instability (16). Rates of CI-AKI are reduced by ensuring patients are well hydrated, avoiding patients at high risk of CI-AKI, avoiding the use of concomitant nephrotoxins, and avoiding hyperosmolar contrast agents (12). Despite this, CI-AKI is still one of the commonest causes of AKI in hospitalized patients (135), and to date no therapeutic interventions have been shown to reduce the incidence of CI-AKI (173).

Pathophysiology of CI-AKI. Contrast agents are freely filtered and concentrated in the collecting ducts and distal tubules as water and low-molecular weight solutes are preferentially reabsorbed (139). Increased concentrations of contrast agents in the glomerular filtrate may account for the higher rate of CI-AKI with intra-arterial vs. intravenous contrast agents. Animal studies indicate that there is an early reduction in renal medullary blood flow giving rise to tubular ischemia (140). It is unclear whether this is a direct effect on the renal vasculature or an indirect effect resulting from tubuloglomerular feedback (89). In addition to these vascular effects, in vitro studies using cultured tubular epithelial cells and ex vivo studies using isolated tubules indicate that contrast agents may also cause direct renal tubular toxicity associated with increased production of pathogenic reactive oxygen species (12). Histological studies in patients with CI-AKI are limited to isolated case reports, so it is unclear whether the pathophysiological mechanisms of renal injury reported in animal and in vitro models actually occur in humans. However, cases in which renal pathology has been reported do show evidence of tubular injury (85). Additional confounding factors may contribute to the pathophysiology of renal injury in patients with CI-AKI, including the possibility of atheroembolization to the kidney from intra-arterial manipulations (106).

Clinical trials and preclinical models for CI-AKI. All of the clinical trials we identified evaluated pretreatment interventions for CI-AKI (Table 1). The incidence of CI-AKI in these studies was relatively low (1–15%) despite selecting for patients with diabetes mellitus and/or mild to moderate CKD (7, 75, 76, 160). Most of the published studies were performed in patients treated with intra-arterial contrast agents at the time of percutaneous coronary intervention (7, 46, 76, 160). One study was conducted exclusively in patients treated with intravenous contrast agents at the time of computed tomography imaging (75). This study failed to show an effect because none of the
patients developed AKI despite selecting for patients with preexisting CKD. Primary end points for all of these studies were based on short-term changes in serum creatinine. Benefits for long-term outcomes such as CKD and mortality were not assessed. Since clinical trials require large patient cohorts to identify changes in long-term CKD and mortality outcomes, there is an even greater need that the preclinical models of CI-AKI accurately mimic human CI-AKI. Of the animal studies used to evaluate the therapeutic interventions in patients with CI-AKI summarized in Table 1 [sodium bicarbonate, NAC, iron chelators (deferoxamine), and vitamin E], only 3 of 23 were performed using animal models of CI-AKI (Table 2) (80, 166, 178). The majority of animal studies were performed using IR-AKI, sepsis, or toxin models of AKI. This may reflect the paucity of animal models of CI-AKI that model the clinical scenarios commonly seen in patients. For example, of the preclinical models of CI-AKI used, one study protocol involved water deprivation before injection of the contrast agents (178), while another utilized high-osmolality contrast media (166), neither of which reflect current clinical practice in patients treated with contrast agents (12). Furthermore, only one-third of the preclinical CI-AKI studies that were performed used intra-arterial contrast injections (80), and none were performed in animals with preexisting CKD or diabetes. Despite these limitations, it is likely that these models of contrast-induced renal injury more closely mimic the pathophysiology of CI-AKI than currently utilized IR-AKI, sepsis, and toxin models.

**CP-AKI**

Platinum-based chemotherapies, of which cisplatin is the most commonly used and effective agent, have been in use for nearly 40 years and are important agents in the therapeutic arsenal for a variety of solid organ malignancies in adults, including head and neck, lung, testis, and ovarian and bladder cancer, as well as childhood osteosarcomas and hepatoblastomas (65, 171). The principal dose-limiting side effect of cisplatin is AKI (115, 136). Many patients who develop AKI are managed by their oncologist and do not see a nephrologist unless renal replacement therapy is required or severe metabolic complications, such as severe magnesium wasting, occur (136). Despite the use of standard precautions to ensure patients are well hydrated, do not have preexisting CKD, and avoiding the use of concomitant nephrotoxins, up to 30% of patients still develop AKI (136). The repeated dosing requirements for the majority of cisplatin-containing chemotherapy regimens carries a significant risk of CKD (136). Development of CKD is of particular concern for long-term follow-up in children treated and cured of their malignancy using cisplatin chemotherapy regimens (77). In patients who develop significant renal impairment after cisplatin treatment, increased mortality is observed due to markedly increased rates of tumor recurrence (24). Since clinical guidelines require reduced or missed doses of cisplatin if serum creatinine does not return to baseline (81), increased rates of tumor recurrence in patients with CP-AKI may result from a failure to complete the chemotherapy regimens. Therefore, the effectiveness of chemotherapy is an important consideration for outcomes of therapeutic trials of CP-AKI since, unlike other indications, therapeutically successful an AKI intervention study might be judged by its ability to ensure completion of chemotherapy.

**Pathophysiology of CP-AKI**. The renal toxicity of cisplatin has been studied in vitro and in rodents and results from the concentration of cisplatin in the proximal tubular epithelium as a result of the selective uptake of cisplatin by organic cation transporters (50). Once in the cells, cisplatin induces DNA damage and activates a variety of signaling pathways that promote apoptosis as well as spontaneous necrosis and programmed necrosis (115, 136). Limited data on human tissues obtained from autopsies show dominant proximal tubular degeneration, necrosis, and repair, but also damage to distal tubules and the collecting duct epithelium (158).

**Clinical trials and preclinical models for CP-AKI**. We were only able to identify a single, small, completed clinical trial of a therapeutic intervention for prevention of CP-AKI after cyclical treatment with cisplatin (listed in www.clinicaltrials.gov) (Table 1). No reduction in AKI was observed in patients pretreated with the antioxidant silymarin (142). This clinical study was supported by one preclinical study that used a single dose of cisplatin in rats pretreated with silymarin (Table 2). Treated rats showed less tubular injury at 2 wk, although functional markers such as creatinine or blood urea nitrogen were not assessed (3). While this study is not representative of the breadth of preclinical studies that have been used to study CP-AKI (115), it does reflect a number of common weaknesses. Nearly all of the preclinical studies to date have been performed in mice or rats after a single high dose of cisplatin (from 5 to 30 mg/kg) (49, 114, 149). These models contrast with standard cisplatin treatment regimens in patients in whom the drug is usually administered at multiple lower doses (60–100 mg/m²) at 3- to 4-wk intervals for several months (136). To model this, the Siskind laboratory (144) recently developed a repeated dosing model in mice with administration of 7 mg/kg cisplatin once a week for 4 wk. An advantage of this repeated dosing model is that mice are able to survive the course of cisplatin treatment and can be aged out to examine the long-term effects on kidney pathology and function. In addition to differences in dosing regimens, while the majority of adult patients with head and neck and lung cancer are elderly, we are unaware of preclinical studies that have been performed in older rodents or in rodents with CP-AKI-associated risk factors, including CKD and diabetes.

The dearth of clinical trials for CP-AKI likely reflects the competing end points of preventing AKI and reducing tumor growth. A number of the drugs tested to prevent CP-AKI might also promote tumor growth directly or might inhibit chemotherapeutic efficacy. For example, while numerous studies have tested the efficacy of erythropoietin in preventing CP-AKI (39), erythropoietin use in patients with cancer is controversial since there is concern that erythropoietin may increase tumor growth (38). Other treatments that prevent CP-AKI in animal models, including inhibition of caspases and anti-TNF therapy (49, 128), might also limit the therapeutic efficacy of cisplatin against tumors. Despite this, we are aware of only two studies that have examined the dual effect of therapy on both CP-AKI and tumor growth (116, 129). The study by Ravichandran et al. (129) is a sobering reminder that the model of injury studied can result in very different results. In that study, the role of CD4 T cell depletion in the prevention of CP-AKI was examined in a CP-AKI model that was developed in tumor-
bearing mice. Cisplatin administered weekly for 4 wk increased serum creatinine and caused tumor shrinkage by 2 wk. Although depletion of CD4 T cells protects against CP-AKI in the high dose, single injection model, in the 4-wk tumor model CD4 T cell depletion did not protect against CP-AKI and also reduced the effectiveness of cisplatin to shrink tumors: tumors were larger in the CD4-depleted mice treated with cisplatin. The results of this report highlight two critical issues in future cisplatin-induced AKI work: 1) results in the more clinically relevant low dose 4 wk model may not be consistent with results in the high-dose short-term models; and 2) measures that may protect against AKI may adversely affect tumor growth.

SA-AKI

SA-AKI is probably the most challenging and complex clinical scenario for studying therapeutic efficacy in AKI. AKI is a frequent complication of sepsis, occurring in >50% of patients with sepsis in intensive care units (62). SA-AKI is an independent predictor of mortality and progressive CKD, particularly in patients who require dialysis (14, 62). The incidence of sepsis has been progressively increasing over the last 20 years. This has been attributed to increased population age, survivable chronic diseases, therapeutic and human immunodeficiency virus-associated immunosuppression, and increasingly complex surgical interventions (97). Patients with SA-AKI usually present with a rapid decline in renal function after the onset of sepsis, but the clinical course of patients with sepsis is highly variable. For example, sepsis is associated with activation of a systemic inflammatory response during the acute illness, yet over time, a compensatory anti-inflammatory response may develop which predisposes patients to secondary infections (26). Patients may therefore present with a primary infection such as postoperative sepsis after bowel surgery, followed by more severe sepsis after they develop a secondary infection such as a hospital-acquired pneumonia (30). Other factors may complicate the diagnosis since sepsis may be treated with nephrotoxic antibiotics, and patients with AKI from other causes, such as CI-AKI and CSA-AKI, are also at increased risk of sepsis (94). AKI-specific risk factors in sepsis are shared with other causes of AKI and include preexisting CKD, hypertension, and diabetes mellitus (62).

Pathophysiology of SA-AKI. SA-AKI is part of a complex clinical syndrome in which a profound systemic inflammatory response syndrome (SIRS) is activated in response to an infection (8). Organ dysfunction results from reduced oxygen delivery to and/or usage by tissues. Reduced oxygen delivery may result from reduced blood pressure in septic shock, as well as abnormalities in microcirculatory blood flow resulting from reduced red blood cell deformability, coagulopathy associated with disseminated intravascular coagulation, and endothelial dysfunction (8). In addition to reduced tissue oxygenation, hyperactivation of the innate immune response by activation of pathogen associated molecular pathways (PAMPs) gives rise to pathogenic oxidative stress and further tissue injury in sepsis. Activation of death-associated molecular pathways (DAMPS) in neighboring cells further enhances inflammation (8, 174). Despite these effects on tissue oxygenation, oxidative stress, and inflammation, autopsy series from patients with SA-AKI dying of severe sepsis fail to detect widespread cellular necrosis in the kidney (157). One explanation for this is that sepsis promotes mitochondrial damage in renal tubular epithelial cells that in turn impairs oxygen usage (163). This may make the tubular epithelium resistant to the effects of reduced oxygen delivery in sepsis, and would explain why there is evidence of widespread structural abnormalities in mitochondria and increased mitophagy in the kidneys of patients dying with SA-AKI (157).

Clinical trials and preclinical models for SA-AKI. There have been a large number of completed and ongoing therapeutic trials in patients with SA-AKI (Table 1) (8, 154). None of these therapeutic interventions have been translated into clinical practice (37). A common weakness in clinical study designs for SA-AKI has been the failure to identify and select phenotypic subgroups of patients that are predictive of adverse outcomes (34). Given the heterogeneous pathophysiology of SA-AKI, in the absence of subgroup selection clinical studies may either show negative results or have to recruit prohibitively large numbers of patients to see significant treatment effects. In addition, when clinical studies are planned there has often been a failure to carefully consider how timing of the intervention in patients relates to timing of intervention in animal studies. For example, a commonly cited example of the failure of animal studies to translate to patients with sepsis is the failure to effectively translate anti-TNF therapy, which is beneficial in animals with sepsis, to patients (4). However, the clinical studies used anti-TNF therapies in patients with established sepsis whereas the beneficial effects of anti-TNF in animal models of sepsis required administration before the onset of the sepsis (58, 162). In a recent study from the Faubel laboratory (23) using both the cecal ligation and perforation (CLP) and endotoxin models of sepsis, it was determined that the onset of AKI occurs rapidly in the course of sepsis and that prophylactic administration of TNF antibodies protected against AKI. However, anti-TNF therapy administered 2 h after sepsis did not protect against AKI. Thus the failure in the clinical trials that administered anti-TNF therapy in established sepsis is consistent with these animal data, indicating that anti-TNF therapy is ineffective in established disease.

A number of different preclinical models of severe sepsis and SA-AKI have been developed in rodents and larger mammals (45, 165). Of the animal studies used to evaluate therapeutic interventions for patients with SA-AKI summarized in Table 1 [sodium bicarbonate, erythropoietin analogs, hydroxyethyl starch (HES), and insulin], the majority were based on nonsepsis models of AKI (Table 2). It can be argued that models such as IR-AKI, glyceral-induced rhabdomyolysis, and cardiopulmonary bypass-associated-AKI (CPB-AKI) model some of the alterations in renal hemodynamics and inflammatory responses seen in SA-AKI (8, 56, 60, 64). However, it is unlikely that these preclinical models share all of the pathophysiological features of SA-AKI. Only 1 of 15 preclinical studies identified in our search used the rat cecal ligation and puncture (CLP) model of SA-AKI to evaluate efficacy of therapeutic interventions for the prevention of SA-AKI (131). Failure to utilize sepsis models of SA-AKI is probably a practical issue reflecting difficulties establishing some of the more clinically relevant models of SA-AKI, such as the rat CLP model, in which fluid resuscitation and antibiotic therapy are used to mimic SA-AKI in patients (45). However, there are limitations even to these more clinically relevant models of...
SA-AKI. For example, CLP AKI in rodents is rarely studied for >24 h and yet patients with SA-AKI are often septic for several days. In addition, SA-AKI is associated not only with polymicrobial, bowel-associated sepsis but also with lung, intravascular, and urogenital sepsis (14). Apart from intravenous or intra- peritoneal inoculation with bacteria (45), we are unaware of SA-AKI models that have used alternate sites of primary infection. In addition, the bacterial species may affect therapeutic responses to intervention. For example, pretreatment with TNF antibodies confers a marked improvement in survival after intravenous injection of Escherichia coli but has limited survival benefit after intravenous injections of two other gram negative bacteria, Pseudomonas aeruginosa and Klebsiella pneumoniae (147). While these studies did not evaluate effects on SA-AKI, they illustrate the importance of evaluating therapeutic efficacy for SA-AKI induced by different pathogens. Finally, there is evidence that preexisting CKD increases the severity of AKI and worsens survival in the rat CLP model (44). Importantly, preexisting CKD can also alter therapeutic responses in sepsis. For example, rats that have undergone ½ nephrectomies show reduced survival benefit of anti-vascular endothelial growth factor (VEGF) therapy after CLP but improved survival after anti-high-mobility box group protein 1 (HMGB1) treatment (82).

Additional Considerations

Pharmacokinetics and target engagement studies. Differences in therapeutic responses between mammalian species may result from differences in drug metabolism and dosing, and it is often unclear whether doses that are administered achieve effective tissue concentrations of the drugs. For example, of 39 preclinical studies we identified, none included pharmacokinetic studies and only 4 included dose-response analyses (Table 2). An alternative approach is to evaluate whether the molecular pathways being targeted are efficiently engaged by the therapeutic intervention. Fourteen of thirty-nine studies evaluated target engagement in the kidney by the therapeutic intervention: 13 of these evaluated the effects of antioxidants on markers of oxidative stress in the kidney (Table 2). Since 20 of 39 of the studies evaluated effects of antioxidants in AKI, this suggests that while target engagement analysis is commonly performed in preclinical antioxidant studies, it is rarely performed for other therapeutic interventions. This is likely because oxidative stress markers are relatively easy to measure while the therapeutic targets engaged by other therapeutic interventions, such as erythropoietin, insulin, and brain natriuretic peptide, are poorly defined and difficult to assess.

Sex and genetic heterogeneity. The majority of preclinical studies in AKI are performed in males because renal injury is generally more severe and models are less affected by hormonal fluctuations that occur in females during the estrus cycle (39, 119). However, while female mice are protected from IR-AKI, they have increased susceptibility to CP-AKI (172), and importantly may have different responses to therapeutic interventions after AKI. For example, while male rats are protected from CP-AKI after treatment with erythropoietin, erythropoietin has no protective effects on CP-AKI in females (48). Despite this and consistent with the published literature (39), only 4 of 39 of the preclinical studies that we identified performed studies in females (36, 84, 109, 178), and only 2 of these studies compared effects in males and females (84, 109). Humans are also genetically heterogeneous, and the genetic heterogeneity has been shown to affect the severity of AKI in both mice and rat models of IR-AKI (18, 90). Interestingly, the majority of the preclinical AKI drug efficacy studies we identified were performed using outbred, genetically heterogeneous rats (mostly Sprague-Dawley and Wistar). However, all four of the mouse preclinical AKI studies that we identified were performed using inbred, genetically identical mouse strains (Table 2).

Recommendations

Our inability to translate preclinical discovery into effective therapeutic interventions that improve clinical outcomes in patients with AKI suggests that our current approaches are flawed. While this is clearly a complex problem with deficiencies in clinical and preclinical study design, models, and approaches, we suggest it is an opportune time to take a few steps back and reexamine current practices. Our analysis of the preclinical models that have been used to support recent clinical efficacy studies for AKI have identified a number of common limitations that we feel could now be addressed.

Better modeling of comorbidities and risk factors. The presence of comorbidities that affect the severity of AKI may affect responses to therapy. In addition to the effects of CKD on therapeutic responses in sepsis (82), another example of a comorbidity that may reduce therapeutic responsiveness in AKI is the reduced regenerative capacity of the aging kidney (2). Additional comorbidities, including CKD and diabetes, may affect therapeutic responses. For example, clinical studies of remote ischemic preconditioning (RIPC) may prove to be ineffective in clinical trials because many of the risk factors used to select patients for intervention also reduce the protective effects of RIPC after AKI (98). Other variables include the effect of gender on severity of injury and therapeutic responses after AKI (48, 119, 172), as well as genetic (18, 88) and environmental heterogeneity, including the effects of polypharmacy, which are particularly common in elderly patients with AKI (133). Future studies should focus on developing more complex models of AKI in older animals, both males and females, as well as studies in animals with preexisting CKD, diabetes, and should also evaluate the effects of commonly used polypharmacies on these therapeutic responses. In addition, while many of the preclinical studies in rats are performed using outbred, genetically heterogeneous strains, efforts should be made to study outbred strains in mouse studies as well.

Incorporating dose-response, timing, and target engagement studies. Some attempt should be made to determine the optimal dosing and timing for preclinical therapeutic intervention studies. While many laboratories may not have the ability to perform pharmacokinetic studies, analysis of dose-response effects are likely to capture effects of inadequate dosing on drug efficacy. If the molecular target of the therapeutic intervention is known, efforts should be made to evaluate activity and/or expression of the target in treated mice to determine whether effective concentrations of the drug are reaching the target organ. Finally, while many preclinical studies have focused on prevention as a therapeutic goal, attempts should be made to compare effects of preventive treatment with delayed...
treatment regimens on AKI outcomes. By clearly defining both effective dosing and timing of therapeutic interventions, preclinical therapeutic efficacy studies can be better used to direct clinical study design in the future.

**Better modeling of human disease.** As a guiding principal, we recommend that attempts be made to test therapeutic interventions in preclinical models that reflect the nature of renal injury that occurs in patients with AKI. For example, a drug that is being evaluated in patients with CI-AKI should also be tested in animal models of CI-AKI, while drugs being evaluated in patients with SA-AKI should be tested in valid models of SA-AKI. Our review indicates that this has not always been the case. It remains to be seen whether additional cost and time spent modeling more complex and varied clinical scenarios, such as the use of CPB in rodents and pigs to model CSA-AKI, or the use of intra-arterial contrast agents to model CI-AKI, will allow for the first convincing translation of a preclinical AKI drug discovery to patients. Finally, where effects of AKI interventions might adversely influence a concurrent disease process, such as the use of erythropoietin in patients with cancer undergoing cisplatin therapy, we recommend that attempts are made to evaluate effects of these therapeutic intervention on the progression of the associated disease in animal models before these interventions are tested in humans.

**Improved understanding of the cellular and molecular pathways in human AKI.** There is a scarcity of renal biopsy material available to interrogate molecular pathways that are involved in the pathogenesis of human AKI. As a result, it is unclear whether the pathways being targeted in preclinical studies are relevant to human disease. This problem has received scant attention in the AKI literature, but a number of sobering examples of this can be found in the non-AKI literature. For example, while some mouse models of diabetic nephropathy recapitulate the pathological findings and progression of the human disease, only a minor subset of pathways that are activated in these preclinical models are activated in renal biopsies from patients with diabetic nephropathy (22, 59). There is also a divergence of the molecular pathways regulating inflammatory responses in mice and humans. This has precipitated a vigorous debate about the relevance of mouse models to the study inflammatory pathologies, including sepsis, in humans (141, 155, 156, 170). These concerns might be mitigated with the development of “humanized” mouse models, in which human genomic loci are transferred into the mouse genome (27, 42). However, for these models to be valid there is still a need to understand which pathways are involved in the pathogenesis of human disease. These questions underscore the need to develop a better understanding of the similarities and differences between the molecular pathogenesis of AKI in animal models and humans. In the long term, our ability to assess this is dependent on developing a deeper understanding of the comparative molecular pathogenesis of human and experimental AKI. This will only be possible if we are able to develop renal biopsy tissue repositories in patients with AKI. While waiting for this, we suggest preclinical efficacy studies are performed in multiple models of AKI: drugs that are effective in multiple models of AKI, particularly if they are effective in males and females and in different species, are more likely to be effective patients with AKI in which the pathophysiologies are often complex and multifactorial.

**Conclusions**

The lack of effective therapies for patients with AKI represents an important and unmet medical need. While there are many explanations for this, ranging from deficiencies in clinical study design, patient selection, and outcomes measures, the development of preclinical models that are predictive of therapeutic responses in patients with AKI is a challenge that can and should be addressed. This will take time and effort, and in all likelihood will increase the cost of preclinical AKI research. However, the cost of developing preclinical models which are predictive of therapeutic efficacy in patients with AKI pale compared with the costs of funding futile clinical trials. While recommendations in this review reflect the authors’ opinions, we hope that these recommendations will provide a springboard for further discussion that will lead to the development of consensus guidelines for the nephrology research community. In the long term, this would serve as the foundation for collaborations and commitment from interested stakeholders in industry, academia, and the research funding agencies that will be required to support the development and application of these models more widely by the nephrology research community.

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**AUTHOR CONTRIBUTIONS**

Author contributions: N.I.S. and M.P.d.C. prepared figures; N.I.S. drafted manuscript; N.I.S., L.J.S., S.F., and M.P.d.C. edited and revised manuscript.

**REFERENCES**


125. slev J, Group ST; Scandinavian Critical Care Trials Group. Hy-
136. Roncal CA, Mu W, Croker B, Reungjui S, Ouyang X, Tabah-Fisch I, Johnson RJ, Ezzia AA. Effect of elevated serum uric acid on cisplatin-
155. Sleeman P, Patel NN, Lin H, Walkden GJ, Ray P, Welsh GJ, Satchell SC, Murphy GH. High fat feeding promotes obesity and renal inflam-
156. mation and protects against post cardiopulmonary bypass acute kidney injury in swine. Crit Care 17: R262, 2013.
162. Thakar CV, Worley S, Arrigain S, Yared JP, Paganini EP. New strategy of alpha- and gamma-tocopherol to prevent contrast-induced acute kidney injury and neutrophil gelatinase-associated lipocalin in patients undergoing cardiac surgery: a randomized, double-blind con-
164. Tasanarong A, Duangchana S, Somunsurp S, Homvises B, Satt-
165. habudha O. Prophylaxis with erythropoietin versus placebo reduces acute kidney injury and neutrophil gelatinase-associated lipocalin in patients undergoing cardiac surgery: a randomized, double-blind con-


