Modeling human disease: a mouse model of acute kidney injury to chronic kidney disease progression after cardiac arrest

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Matsushita *et al.* describe a model of acute kidney injury to chronic kidney disease progression in mice surviving cardiac arrest: mice develop severe acute kidney injury that initially recovers but is followed by the onset of impaired renal function on longer-term follow-up. These findings suggest that distinct cardiorenal toxicities and/or injury dynamics are operative in this cardiac arrest model that do not occur in traditional models of acute kidney injury, providing new opportunities for therapeutic and biomarker discovery for an important clinical problem.

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Acute kidney injury (AKI) is a substantial cause of morbidity and mortality, with more than 1 million patients in the United States affected annually. Clinical treatment for AKI primarily focuses on hemodynamic optimization, eliminating risk factors for additional injury and providing renal replacement therapy if necessary. Despite a sizeable literature on the basic science of AKI, the absence of specific medical therapies for the treatment of AKI is a major shortcoming in the field of nephrology. This has led to increased attention on optimizing preclinical studies to make them more clinically relevant, thereby decreasing barriers that have hindered transition from preclinical discovery to clinical treatment.[1] This has led to a push for AKI studies to use models that better reflect injury seen in patients. Because AKI is a major risk factor for the development of chronic kidney disease (CKD), models that evaluate long-term renal outcomes resulting from the transition of injury from AKI to CKD provide additional insight into an important clinical problem.

Matsushita *et al.* (2019) recently reported a novel murine model of AKI to CKD progression after cardiac arrest and cardiopulmonary resuscitation (CA/CPR),[2] a commonly encountered clinical scenario that is a major cause of AKI.[3] Their group has previously shown that the CA/CPR model in mice leads to AKI 24 hours post-arrest, as determined by decreased glomerular filtration rate (GFR) and elevated serum creatinine.[4] In the current study, the authors use the same model, but for the first time carefully describe unexpectedly dynamic changes in renal functional recovery, tubular injury, inflammation, and fibrosis that occur over a 7-week period after the initial cardiac arrest. In their model, mice are anesthetized with isoflurane, intubated and ventilated, and cardiac arrest is induced with potassium chloride. Animals remain asystolic for 8 minutes, after which CPR is performed by finger chest compressions, at a rate of approximately 300 compressions per minute, and epinephrine is administered, after an advanced cardiac life support–like protocol. CA/CPR animals sustained AKI with near-absent GFR at 24 hours post-arrest. GFR recovered 2 weeks later, but at 7 weeks, post-arrest animals demonstrated reduced GFR, elevated blood urea nitrogen, and renal fibrosis, suggestive of CKD. Even during the interval recovery of GFR, they show evidence of ongoing renal inflammation and injury from the time of arrest to their final endpoint (Figure 1).

This long-term CA/CPR model is technically challenging and, because of the high mortality rates encountered over the first week after cardiac arrest (average mortality of 47.1 ± 14.1% from the 3 cohorts of mice in the article), definitive hypothesis testing experiments will require investigators to use larger numbers of mice than are typically used for toxin or ischemia reperfusion-induced AKI studies. However, as a clinically relevant model of AKI progressing to CKD after surviving cardiac arrest, this model holds promise for identifying mechanisms that may be amenable to therapeutic intervention. Because this clinical scenario provides a definitive event in time subsequent to which renal injury occurs, the administration of any potential therapeutics could be precisely timed relative to arrest time and integrated with post-arrest protocols.

Their long-term model of CKD progression after cardiac arrest also provides a system to identify biomarkers that will predict which patients are most likely to progress to CKD after cardiac arrest, because only a subset of these patients progress to CKD.[3] CA/CPR mice have complete recovery of GFR at 2 weeks, but tubular injury, kidney injury molecule 1 positivity, and inflammatory cell infiltrates persist at 24 hours, 1 week, and 7 weeks post-arrest. This suggests that recovery of GFR, signified clinically by normalization of serum creatinine, is inadequate to predict

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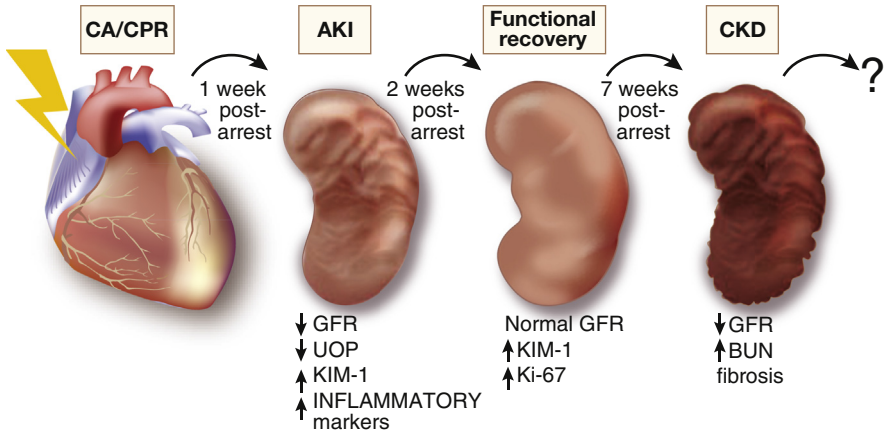

Figure 1 | Modeling the kinetics of acute kidney infection (AKI) to chronic kidney disease (CKD) progression after surviving a cardiac arrest. BUN, blood urea nitrogen; CA/CPR, cardiac arrest and cardiopulmonary resuscitation; GFR, glomerular filtration rate; KIM-1, kidney injury molecule 1; UOP, urine output.

which patients are at risk for progressing to CKD. Several biomarkers, including kidney injury molecule 1 and neutrophil gelatinase-associated lipocalin, have been proposed as alternatives to serum creatinine because they have higher specificity and sensitivity to predict adverse AKI outcomes in other clinical scenarios.[5] This model provides a new approach to identify biomarkers that predict which patients with cardiac arrest are most likely to progress from AKI to CKD, and may therefore require more intense treatment and long-term observation post-arrest.

Perhaps the strongest physiological component of this model is that it links 2 vital organs, the heart and the kidney. The authors use primary cardiac dysfunction as a catalyst for renal injury, a clinical scenario referred to as cardiorenal syndrome (CRS). CRS is a descriptive term that applies to any situation in which cardiac and renal dysfunction coexist. This is an important clinical scenario that is commonly encountered and leads to substantial healthcare burden. A CRS classification was proposed by Ronco and colleagues more than 10 years ago and is largely based on chronicity of injury and organ of primary dysfunction.[6] Their broad definition of 5 CRS subtypes was proposed to highlight the bidirectional relationship between these 2 organs, but the optimal clinical application and mechanistic utility of this classification can be unclear.

Matsushita *et al.* use their model to investigate type 1 CRS, the scenario in which acute cardiac dysfunction causes acute renal dysfunction.[2] Their claim that they are modeling AKI to CKD progression would also suggest this is a model of progression from type 1 to type 2 CRS (i.e., chronic cardiac dysfunction leading to chronic renal dysfunction). Hemodynamic changes, including decreased forward flow and venous congestion, are often implicated as the predominant factors driving renal dysfunction in acute heart failure. However, the authors report relatively severe AKI, beyond what would have been expected after an equivalent 8- to 10-minute renal pedicle clamp time after surgically induced ischemia-reperfusion-induced AKI. This suggests there are pathogenic differences in this model extending beyond mere renal ischemia. Indeed, Wakasaki and colleagues recently reported a possible nephrotoxic role for a circulating factor of cardiac origin, cardiac LIM protein, in post-cardiac arrest CRS.[7] Future studies using this model will be able to further elucidate these bidirectional cardiorenal signaling pathways.

Although there is great potential for this model, several caveats related to this study should be noted. First, post-arrest animals were only followed up for 7 weeks. Although there is no clear definition of CKD in mice, humans are given 3 months to recover from AKI before they are considered to have CKD. Because several markers of injury, including kidney injury molecule 1 expression, were improving at 7 weeks, the possibility that the animals were undergoing a prolonged renal recovery and would return to baseline over the subsequent weeks cannot be excluded. Following up with these mice beyond 3 months in the future will answer this question. Second, the pattern and timing of renal injury are observed in the setting of healthy, relatively young mice. This scenario is not typically observed clinically because the majority of patients sustaining cardiac arrest are older and more commonly have some baseline cardiac and/or renal dysfunction. Although this study demonstrates what occurs in the setting of normal kidneys, one should be cautious about generalizing these data to the diseased substrate. An earlier study from the Hutchens lab demonstrated that middle-aged male (but not female) C57Bl/6 mice (43–48 weeks) developed more severe AKI 24 hours after CA/CPR than younger mice (10–15 weeks).[8] Further studies are needed to determine whether differences in short-term renal outcomes in older male versus female mice are predictive of long-term CKD progression after CA/CPR. Thirdly, the authors achieved return of spontaneous circulation in 87%

of their animals, which is higher than is typically observed in patients after cardiac arrest, particularly with non-shockable rhythms. Furthermore, they had approximately 50% survival post-CPR for all groups, which is a substantial mortality rate, but much lower than the 80% to 90% mortality rate before hospital discharge seen in patients.[9] This is likely to be related to the degree of monitoring under experimental conditions, though an underlying physiological difference between species cannot be excluded.

Overall, this model provides an opportunity to investigate the basic science of cardiorenal syndrome with the hope of developing new treatments for renal disease in patients with cardiac arrest. By modeling unique pathophysiological events that do not occur in traditional models of AKI, we also anticipate that the long-term CA/CPR model, although technically challenging, is likely to lead to other clinically impactful discoveries including exploration and identification of novel cardiorenal signaling events that enhance renal injury after cardiac arrest, and discovery of novel biomarkers that are predictive of which patients are likely to develop AKI and CKD progression.

DISCLOSURE
All the authors declared no competing interests.

REFERENCES

1. 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*. 2016;310:F972–F984.
2. Matsushita K, Saritas T, Eiwaz MB, et al. The acute kidney injury to chronic kidney disease transition in a mouse model of acute cardiorenal syndrome emphasizes the role of inflammation. *Kidney Int*. 2020;97:95–105.
3. Tujjar O, Mineo G, Dell'Anna A, et al. Acute kidney injury after cardiac arrest. *Crit Care*. 2015;19:169.
4. Ikeda M, Wakasaki R, Schenning KJ, et al. Determination of renal function and injury using near-infrared fluorimetry in experimental cardiorenal syndrome. *Am J Physiol Renal Physiol*. 2017;312:F629–F639.
5. Wasung ME, Chawla LS, Madero M. Biomarkers of renal function, which and when? *Clin Chim Acta*. 2015;438:350–357.
6. Ronco C, House AA, Haapio M. Cardiorenal syndrome: refining the definition of a complex symbiosis gone wrong. *Intensive Care Med*. 2008;34:957–962.
7. Wakasaki R, Matsushita K, Golgotiu K, et al. Glomerular filtrate proteins in acute cardiorenal syndrome. *JCI Insight*. 2019;4:e122130.
8. Ikeda M, Swide T, Vayl A, et al. Estrogen administered after cardiac arrest and cardiopulmonary resuscitation ameliorates acute kidney injury in a sex- and age-specific manner. *Crit Care*. 2015;19:332.
9. Goldberger ZD, Chan PS, Berg RA, et al. Duration of resuscitation efforts and survival after in-hospital cardiac arrest: an observational study. *Lancet*. 2012;380:1473–1481.

Sirtuin 6 and renal injury: another link in the β-catenin chain?

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A protective role for sirtuin 6 (Sirt6) in the context of chronic renal injury is reported by Cai *et al.* in this issue of *Kidney International*. The mechanism is thought to be mediated by Sirt6's deacetylase activity, specifically on β-catenin target genes. This commentary discusses these results and the interaction between Sirt6 and β-catenin within the broader context of β-catenin signaling and injury.

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Chronic kidney disease (CKD) is a growing problem, affecting up to 10% of the global population, and tubulointerstitial fibrosis is the pathological hallmark of CKD. Persistent renal tubule injury leads to tubule atrophy, reduced function, and production of profibrotic growth factors and inflammatory cytokines. These growth factors can activate neighboring fibroblasts/pericytes to become myofibroblasts, the major producers of extracellular matrix components that comprise fibrosis. Treatments to halt the progression of injury to CKD and end-stage renal disease would ideally promote epithelial repair and reduce extracellular matrix production, but no such therapies are currently available.

Thus, there is a huge unmet medical need for new therapeutic strategies to halt CKD progression and tubulointerstitial fibrosis. Sirtuins, a class of nicotine amide dinucleotide (NAD+)–dependent deacetylases, were originally identified as modulators of lifespan in yeast but also have been shown to modulate renal injury. There are 7 mammalian sirtuin isoforms (Sirt1–Sirt7), with Sirt1 being the best-studied in the context of renal injury. Sirt1 can ameliorate acute kidney injury and diabetic kidney disease and reduce fibrosis in the unilateral ureteral obstruction model.[1,2] The mechanisms by which Sirt1 is protective in CKD models include reduction of cyclooxygenase-2 and inhibition of oxidative stress, and targeting of transforming growth factor–beta (TGF-β) signaling, signal transducer and

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