Metabolic Stress Resistance in Acute Kidney Injury: Evidence for a PPAR-Gamma-Coactivator-1 Alpha-Nicotinamide Adenine Dinucleotide Pathway

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Abstract

Acute kidney injury (AKI) is estimated to affect 3–10% of all hospitalized adults in the United States, making it one of the most common inpatient diagnoses. Despite this staggering incidence, most individuals exposed to AKI stressors, such as intravenous radiocontrast or cardiopulmonary bypass, do not develop AKI. In fact, whereas animal models of ischemia, sepsis, or nephrotoxicity suggest near-uniform responses to stressors, the natural history of stressed patients is highly heterogeneous. Recent studies of mitochondrial perturbations underlying experimental and human AKI suggest a conserved metabolic contribution to this variance. The renal

tubule is only second to the heart in terms of mitochondrial abundance, reflecting the exquisite need for fuel combustion to generate the energy for active solute transport. The homeostasis of nicotinamide adenine dinucleotide (NAD⁺), a requisite coenzyme in oxidative metabolism, may be an important determinant of the renal response to AKI stressors. This mini-review highlights recent studies implicating NAD⁺ dysregulation in experimental and human AKI and summarizes findings from a pilot randomized trial to augment NAD⁺ among at-risk individuals.

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Fig. 1. Overview of NAD⁺ metabolism. NAD⁺ is generated within cells from discrete dietary precursors: the essential amino acid tryptophan by a series of enzymes collectively referred to as the “de novo NAD⁺ biosynthesis pathway;” the vitamin B3 analog nicotinamide (also referred to as niacinamide) by the enzyme Nampt; and other precursors that include NA and NR. NAD⁺ is cleaved by enzymes of different classes: sirtuins, which remove acetyl or acyl modifications from proteins; PARPs, which add 1 or more poly-ADP ribose moieties to proteins; and ectonucleotidases such as CD38, which generate cyclic ADP ribose from NAD⁺. NAD⁺, nicotinamide adenine dinucleotide; PARPs, poly-ADP ribose polymerases; NA, nicotinic acid; NR, nicotinamide riboside; Nampt, nicotinamide phosphoribosyltransferase.

Multiple strands of evidence implicate mitochondria at the nexus of renal health and disease. Classic tubular transport studies identify a linear relationship between filtration fraction and oxygen consumption; ultrastructural studies of human acute kidney injury (AKI) have consistently described early mitochondrial swelling in tubular epithelium; and single gene diseases affecting mitochondria exhibit a high prevalence of tubulopathy with progressive renal failure [1]. Thus, biochemical, pathological, and genetic studies all suggest that intact mitochondrial function is essential for normal tubular function, and deranged mitochondria are present in disease.

Along the nephron, the proximal tubule bears the largest burden of active solute transport. This segment preferentially combusts fatty acids to generate adenosine triphosphate. Fatty acid oxidation requires mitochondria and oxygen. Within mitochondria, the coenzyme nicotinamide adenine dinucleotide (NAD⁺) carries high-energy electrons from fatty acid oxidation to the electron transport chain. NAD⁺ is therefore a rate-limiting catalyst for fatty acid oxidation. This means that shortages in NAD⁺ result in impaired energy extraction.

Intracellular NAD⁺ concentrations reflect a balance between biosynthesis from discrete dietary precursors and “consumption” by enzymes that cleave NAD⁺ to release nicotinamide (Fig. 1). Subcellular compartmentalization of NAD⁺ also appears to be important but is be-

yond the current scope [2]. Stressors such as ischemia have been known to induce NAD⁺ consuming enzymes such as poly-ADP-ribose polymerases and CD38; induction of these enzymes lowers NAD⁺ [3]. Tran and colleagues recently reported that renal tubular cell NAD⁺ levels are suppressed in models of AKI and that this reduction of NAD⁺ may blunt fatty acid oxidation, reduce adenosine triphosphate generation, and elevate susceptibility to AKI stressors [4].

Unexpectedly, they found that the stress-related fall in NAD⁺ was not only a consequence of increased consumption but also the result of decreased biosynthesis. In seeking a mechanism of decreased NAD⁺ biosynthesis, this study identified a novel function of the mitochondrial biogenesis regulator PPAR-gamma-coactivator-1alpha (PGC1α) to coordinately induce the enzymes that sequentially convert the amino acid tryptophan to NAD⁺. This series of enzymes is collectively referred to as the “de novo” or “kynurenine” (one of the intermediates between tryptophan and NAD⁺) biosynthesis pathway for NAD⁺. This study showed that renal tubular PGC1α expression defends the nephron against unrelated stressors, and that artificial augmentation of NAD⁺ could effectively mimic PGC1α’s salutary effects in the tubule. Hence, a common PGC1α-NAD⁺ “axis” was described for kidney protection across rodent models of ischemia, inflammation, and nephrotoxicity.

At least 2 biopsy-based studies proposed that human AKI syndromes also exhibit the suppression of PGC1α [4, 5], suggesting that the preclinical AKI findings could be amenable to translational investigation. Since biopsies are not practical for clinical AKI stratification and coactivator proteins such as PGC1α are not attractive pharmaceutical targets, the link between PGC1α and NAD⁺ biosynthesis has suggested new ways to assess energy metabolism and achieve PGC1α-like kidney protection.

Serendipitously, a metabolomics study of murine AKI urine specimens identified a signature highly specific for de novo NAD⁺ biosynthesis, namely, the elevation of urinary quinolinic acid (uQuin) [6]. Quin is a late intermediate in the de novo pathway and has no other metabolic fate other than to become NAD⁺ through the action of quinolinate phosphoribosyltransferase (QPRT) and subsequent enzymes. The elevation of uQuin suggested suppression of QPRT during AKI, consistent with the earlier study linking PGC1α to members of this pathway [4]. Reduced QPRT was then experimentally modeled by gene editing in mice. Strikingly, the urinary metabolome of QPRT haploinsufficient mice recapitulated nearly half of the urinary metabolic changes observed in experimental

post-ischemic AKI. These mice were also highly susceptible to renal ischemia-reperfusion injury. Shortly thereafter, an independent study also identified de novo NAD⁺ biosynthesis as a pathway to fortify the kidney against ischemic and genotoxic stressors [7]. And a third study proposed that, in mammals, only the kidney and the liver exhibit appreciable de novo NAD⁺ biosynthesis [8].

This “reporter” function of uQuin in mice suggested a noninvasive way to monitor the PGC1 α -NAD⁺ axis—more specifically, de novo NAD⁺ biosynthesis—in people. A pilot case control study in cardiac surgery patients followed by a larger cohort study of critically ill patients confirmed that elevated uQuin preceded the clinical diagnosis of AKI [6]. Rising levels of uQuin were monotonically associated with increasing risk of AKI. Together, these results suggested that the perturbation of NAD⁺ biosynthesis observed in the controlled setting of murine ischemia-reperfusion injury was also present in the heterogeneous setting of clinical AKI.

The authors then embarked on a clinical trial to augment NAD⁺ among high-risk patients [6]. Since the de novo pathway appeared to be blocked by QPRT suppression, they chose to administer nicotinamide (also referred to as niacinamide), the base version of vitamin B₃, which the team had shown to boost renal NAD⁺ through another biosynthetic route known as the “salvage” pathway [4]. The design was a Phase I-like pilot study that was randomized and placebo-controlled. The active arms received either 1 or 3 g/day of oral nicotinamide on the day prior to on-pump cardiac surgery, on the day of surgery, and on the first postoperative day. For comparison, the United States recommended a daily allowance of nicotinamide in adults is ~15 mg/day. The results showed that oral nicotinamide elevated circulating (and urinary) levels of nicotinamide. Because serum NAD⁺ may not reflect intracellular levels, the authors were unable to determine whether these doses boosted NAD⁺ itself. Nonetheless, they found no evidence of increased adverse events in the active arms and promising data that suggested nicotinamide-related protection from AKI. The trial was too small and the patient population was too well to detect differences in major renal endpoints, but the results pave the way for future clinical trials of therapeutic NAD⁺ augmentation.

PGC1 α has been studied in the context of tubular cell biology for nearly 15 years [9]. And the first renal genetic mouse model of PGC1 α isolating its benefit in AKI to the tubule was reported almost 9 years ago [10]. But the recent flurry of studies from multiple laboratories elucidating (a) the role of NAD⁺ downstream from PGC1 α ; (b)

the contribution of de novo NAD⁺ biosynthesis to AKI resistance; and (c) the potential for treating AKI with NAD⁺ augmentation should build great excitement for the many fundamental and clinical studies that are now needed [4, 6, 7, 11, 12].

First, what is the set of critical downstream effectors from NAD⁺? A rate-limiting role in energy metabolism is clear, but could NAD⁺ also be modulating cell-regulatory enzymes such as sirtuins to achieve renal benefit? Second, are AKI risk factors such as aging and chronic kidney disease linked by imbalances in NAD⁺ biosynthesis versus consumption? Third, how robust is elevated uQuin as an indicator of waning ability to resist AKI stressors? In what setting(s) is this most important? More broadly, can urinary metabolic signatures enable practical risk stratification in future AKI trials? And finally, can pharmacological manipulations to boost the levels of NAD⁺ find clinical utility in the primary prevention or even treatment of AKI? If so, what are the optimal time windows and clinical contexts? Given that the heart and kidney share a reliance on mitochondrial fatty acid oxidation for normal function, it is also notable that cardiac injury also appeared to be diminished in the above trial, NAD⁺ augmentation. These and many more questions merit investigation. The emerging data linking NAD⁺ balance to AKI resistance and the close conservation of these findings between experimental models and humans propose a new chapter in AKI research.

Disclosure Statement

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