

# The Central Role of Renal Microcirculatory Dysfunction in the Pathogenesis of Acute Kidney Injury

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## Key Words

Renal failure · Microcirculation · Oxygenation · Reactive oxygen species · Nitric oxide

## Abstract

Acute kidney injury (AKI) is a rapidly developing condition often associated with critical illness, with a high degree of morbidity and mortality, whose pathophysiology is ill understood. Recent investigations have identified the dysfunction of the renal microcirculation and its cellular and subcellular constituents as being central to the etiology of AKI. Injury is caused by inflammatory activation involving endothelial leucocyte interactions in combination with dysregulation of the homeostasis between oxygen, nitric oxide, and reactive oxygen species. Effective therapies expected to resolve AKI will have to control inflammation and restore this homeostasis. In order to apply and guide these therapies effectively, diagnostic tools aimed at physiological biomarkers of AKI for monitoring renal microcirculatory function in advance of changes in pharmacological biomarkers associated with structural damage of the kidney will need to be developed.

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Despite the identification of several cellular mechanisms thought to underlie the development of acute kidney injury (AKI), the pathophysiology of the occurrence of AKI is still ill understood [1]. It is clear, however, that instead of a single mechanism being responsible for its etiology, an orchestra of cellular mechanisms in complex interaction with inflammatory, oxidative, and nitrosative factors account for the path leading to renal failure [2–4]. The integrative physiological compartment where these mechanisms come together and exert their deleterious effect is the renal microcirculation [3, 4]. That is why the study of the renal microcirculation and identification of the determinants of its (dys)function in models of AKI are essential for providing insight into the pathogenesis and resolution of AKI. Based on such research, it is expected that translational clinical investigations will provide novel strategies to resolve AKI and equally important new renal monitoring modalities based on physiological markers of AKI to identify and guide these novel therapeutic strategies [5].

The factors responsible for renal microcirculatory dysfunction in advance of AKI include a combination of inflammatory and/or hypoxemic insults that cause activation of leucocytes and/or inflammatory mediators which affect all aspects of normal renal microcirculation essential for the function of the kidney. In this sequence of events, the normal homeostasis between oxygen, nitric

oxide, and reactive oxygen species governing renal signaling, (micro)vascular function, and cellular respiration gets out of balance and the levels of oxygen, nitric oxide, and reactive oxygen species take on a pathogenic role, ultimately leading to renal failure [6]. Renal cellular and subcellular structures essential for renal function are injured by these reactive mediators, leading to lipid peroxidation and the loss of renal tubular cell polarity, as well as altered glomerular filtration.

A main determinant of renal function is the sustained adequate supply and utilization of oxygen at the microcirculatory and mitochondrial level [7]. The highly complex architecture of the renal microvasculature, the need to meet a high energy demand, and the fact that the kidney is borderline ischemic make the kidney an organ highly vulnerable to hypoxic injury. Under normal, steady-state conditions, the oxygen supply to the renal tissues is well regulated in order to meet the oxygen requirements of the kidney to produce ATP through mitochondrial oxidative phosphorylation. The kidney requires a relatively high amount of oxygen to meet its functional needs (approx. 7% of the total body oxygen consumption), most of which is used by the tubular cells for solute reabsorption [8]. However, under pathological conditions, such as during ischemia-reperfusion, shock, or sepsis, the delicate balance of oxygen supply versus demand is disturbed due to renal microvasculature dysfunction [9]. Microcirculatory dysfunction impedes renal microcirculatory flow at the regional level and this leads to the occurrence of hypoxemic pockets especially in the renal cortex, which is highly heterogeneous even following resuscitation procedures [10]. In this context, hypoxemia is regarded as a key characteristic of and contributing factor to AKI [7]. The identification of this hypoxemic component in models of AKI, however, has been elusive due to this heterogeneity and the limitation of tissue oxygenation techniques such as oxygen electrodes which lack the ability to detect this heterogeneity in tissue oxygen levels. Such measurements have missed this hypoxemic component of AKI, resulting in the mistaken interpretation that hypoxemia does not play a role in the understanding of AKI. Techniques, such as histogram analysis of the Pd phosphorescence technique [11] and immunostaining of pimonidazole, able to detect hypoxemic areas in stained histological sections [12, 13] have identified the heterogeneous hypoxemic nature of AKI. This is an important observation because it explains how hemodynamic alterations in the kidney microcirculation, independently of the global renal arterial flow, can sustain an inflammatory state. Here, the heterogeneously present

capillary flow sustains weak microcirculatory units where reperfusion injury at hypoxia-normoxia borders sustains a continuous state of inflammation.

States of reperfusion injury induce leucocyte activation [14, 15] and the generation of reactive oxygen species by enzyme systems such as NADP(H) and xanthine oxidase [16]. These are key pathogenic events which lead to microcirculatory dysfunction that results in AKI. Many experimental studies have shown that therapeutic interventions targeting reactive oxygen species have a favorable course in the prevention of AKI [17]. Examples of such therapeutic interventions include N-acetylcysteine [18] and vitamin C [19]. Intricately associated with oxidative stress is the activation of nitric oxide pathways. Nitrosative stress is mainly associated with the inflammation-induced activation of the inducible nitric oxide enzyme system. Several studies have shown that inhibition of inducible nitric oxide also has a favorable effect on the prevention of AKI. Such compounds have included specific inducible nitric oxide blockade by L-NIL [20, 21] but also more general anti-inflammatory compounds such as dexamethasone [22]. Interactions between the NO system and oxidative radicals fuel the factors responsible for renal injury [23]. An example of the interaction between nitric oxide and reactive oxygen species is the uncoupling of eNOS by the depletion of tetrahydrobiopterin (BH4), which results in the generation of superoxide. Experimental studies supplementing the BH4 precursor sepiapterin have shown such a therapeutic maneuver to be protective in the progression to renal failure [24]. Taking the above information into consideration, we developed an integrative pathophysiological model of AKI whereby inflammatory activation associated with leucocyte activation and endothelial dysfunction interacts with alterations in the homeostasis of the balance between oxygen and reactive oxygen and nitrosative species to fuel the progress to AKI. This integrative pathogenic scenario can be fueled by many factors and acts as a highly toxic hit causing destruction of renal cellular and subcellular structures and opening the door to renal failure [4, 6].

Besides known inflammatory hits such as radiocontrast agents, reperfusion injury, and sepsis contributing to the evolution of cellular and microcirculatory injury which leads to AKI, the inappropriate use of standard therapies in the care of the critically ill can also contribute to renal failure. The main such therapeutic modality concerns the inappropriate use of fluid therapy [25]. Fluid therapy for the treatment of hypovolemia, when administered inappropriately, can promote hypoxemia by ex-

cess hemodilution [26]. Fluids, though effective at promoting tissue hypoperfusion associated with hypovolemia, are poor oxygen carriers and ineffective at promoting tissue oxygenation. We showed this to be the case in a study where we compared the administration of crystalloid resuscitation in a shock model to blood resuscitation. That study showed that, though effective at correcting systemic blood pressure variables, these fluids were highly ineffective at correcting renal tissue oxygenation [27]. In addition, fluids can promote oxidative and inflammatory activation, both of which are deleterious to renal function [28, 29]. The composition of fluids should also be taken into consideration as a contributing factor for AKI. The most used fluid in resuscitation, i.e. 0.9% NaCl, has been especially implicated in this respect and its use as a resuscitation fluid is a subject of controversy [30, 31]. It is clear, however, that the administered volume is the key issue that determines the extent to which fluid therapy is deleterious to renal function. Conventionally used hemodynamic targets are based on systemic hemodynamic variables and it is clear that hitting such targets may result in excessive fluid administration. Thus, a new paradigm is needed for a rationale centered on the true target to correct hypovolemia by fluid administration, which is the correction by fluids of microcirculatory hypoperfusion. It is expected that targeting such a variable will result in less fluid administration and contribute to the reduction of renal failure among critically ill patients [32].

A main contributing factor in the propagation of pathogenic insults leading to AKI has been suggested to be mitochondrial dysfunction. In a unified approach, Gomez et al. [33] identified mitochondrial dysfunction as a key contributor to the development of AKI. Mitochondrial function is critical not only for the production of ATP needed for sodium reabsorption but also because of its role as a prime generator of oxygen radicals during oxidative phosphorylation. Insults associated with the development of AKI, such as sepsis, have been recently shown to inactivate important physiological defense mechanisms such as the presence of manganese superoxide, reducing the endogenous defense capacity of renal cells and promoting oxidative stress [34]. Such increased oxidative stress, besides promoting lipid peroxidation, can in turn be deleterious to mitochondrial function by increasing the levels of mitochondrial uncoupling protein which promotes mitochondrial H<sup>+</sup> leakage, depolarizing the mitochondrial membrane potential needed to drive oxidative phosphorylation and thereby inhibiting mitochondrial ATP production [35].

A central cellular victim of the pathogenic factors leading to AKI is the endothelial cell [36], and its most vulnerable component is one of its subcellular structures called the glycocalyx. The glycocalyx is a thick gel-like (0.2- $\mu$ m) layer coating the vascular lumen side of the endothelial cells of the capillaries. Embedded in the glycocalyx are important molecular systems essential for antioxidant defense and hemostasis as well as signal transduction responsible for vascular (auto)regulation [37]. Reactive oxygen species are one of the main culprits in shedding of the glycocalyx, promoting leucocyte adhesion, compromising the endothelial barrier, and causing glomerular filtration dysfunction [38]. During septic renal failure, the glycocalyx is disrupted by the factors described above, leading to albuminuria [39]. In addition to such structures being destroyed in the processes leading to AKI, transmembrane molecules essential for cellular integrity and communication are also compromised. Such molecules include tight junctions, important for the maintenance of tubular polarity [40], and gap junctions, important for cellular communication essential for the integrative control of renal functions [41].

It is becoming clear that, in order for therapeutic strategies to be effective at resolving AKI, a multimodal approach is needed in which tissue oxygenation is improved by resolution of the heterogeneous hypoxic areas; moreover, control of inflammation is needed and integrative correction of the homeostasis between nitric oxide and reactive oxygen species needs to be established. Although such an integrative therapeutic approach would require several therapeutic interventions, we have shown in experimental studies that a number of drugs have such multimodal therapeutic effects, which is in line with the concepts outlined above. These drugs include: iloprost, dexamethasone, activated protein C, sepratine, and vitamin C [22, 24, 42, 43]. In order to translate these preclinical concepts to critically ill patients in danger of developing AKI, new clinical monitors of physiological biomarkers of AKI will have to be further developed [5].

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