Peritubular Capillary Oxygen Consumption in Sepsis Induced AKI: Multi-parametric Photoacoustic Microscopy

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Abstract

Understanding and measuring parameters responsible for the pathogenesis of sepsis-induced AKI (SI-AKI) is critical in developing therapies. Blood flow to the kidney is heterogeneous, partly due to the existence of dynamic networks of capillaries in various regions, responding differentially to oxygen demand in cortex vs medulla. High energy demand regions, especially the outer medulla, are susceptible to hypoxia and subject to damage during SI-AKI. Proximal tubule epithelial cells in the cortex and the outer medulla can also undergo metabolic reprogramming during SI-AKI to maintain basal physiological status and to avoid potential damage. Current data on the assessment of renal hemodynamics and oxygen metabolism during sepsis is limited. Preclinical and clinical studies show changes in renal hemodynamics associated with SI-AKI and in clinical settings, interventions to manage renal hemodynamics seem to help improve disease outcomes in some cases. Lack of proper tools to assess temporospatial changes in peritubular blood flow and tissue oxygen metabolism are barriers to our ability to understand microcirculatory dynamics and oxygen consumption and their role in the pathogenesis of SI-AKI. Current tools to assess renal oxygenation are limited in their usability as these cannot perform continuous simultaneous measurement of renal hemodynamics and oxygen metabolism. Multi-parametric photo-acoustic microscopy (PAM) is a new tool that can measure real-time changes in microhemodynamics and oxygen metabolism. Use of multi-parametric PAM in combination with advanced intravital imaging techniques has the potential to understand the contribution of microhemodynamic and tissue oxygenation alterations to SI-AKI.

Keywords:

Acute Kidney Injury, Sepsis, hemodynamics, Oxygen metabolism, Photoacoustic microscopy

Pathophysiology of sepsis-induced acute kidney injury (SI-AKI)

Sepsis is described as organ dysfunction resulting from aberrant host response to an infection [1]. Acute kidney injury (AKI) develops in nearly 65% of patient with sepsis and further complicates the therapeutic management of sepsis [2]. Moreover, patients with AKI can also develop sepsis as a consequence [3]. Pathophysiology of SI-AKI is complex and begins with activation of innate immune system mediated by binding of pathogen-associated molecular pattern (PAMPs) (e.g. bacterial endotoxins) or damage-associated molecules (DAMPs) released by host cells (e.g. extracellular ATP, mitochondrial DNA, and other metabolites) to their receptors present in the host cell surface and subsequent release of inflammatory mediators[4]. This evoked immune response then leads to a wide array of pathophysiological changes including dysregulated homeostasis, immunosuppression, and cellular, tissue, and organ dysfunction [5]. Sepsis mediated microcirculatory dysfunction primarily occurs by direct damage to the glycocalyx lining the endothelium of microvessels or by inflammation-induced aberrant vasoreactivity due to release of vasoactive substances by the endothelium, tubule epithelial cells and vascular smooth muscle cells, leading to tissue hypoperfusion, reduced oxygen delivery, and organ dysfunction[6]. Moreover, endothelial activation due to inflammation further leads to leukocyte adhesion and rolling, capillary leakage, interstitial edema, leukostasis and tissue hypoperfusion. In the kidney, sepsis-mediated microcirculatory dysfunction result in local ischemia, tubular dysfunction and injury[7]. At cellular level, mitochondrial dysfunction and reactive oxygen species production during sepsis contribute to tubular cell death[8, 9].

Renal oxygen delivery in normal health

Three distinct types of vascular beds with different flow dynamics are important in the delivery of oxygen and other nutrients to the kidney. These include glomerular capillaries, cortical peritubular capillaries, and medullary vasa recta. RBF in the medulla is significantly lower than the cortex. RBF in the medulla contributes only 10% of the total kidney RBF [10]. Furthermore, different regions of the medulla are also heterogeneously perfused. Compared to the cortex, RBF in the outer medulla is about 40% while the inner medulla is only 10% [11]. Kidneys account for 20% of total oxygen consumption. The kidney cortex has a tissue oxygen tension (PO_2) of ~50mmHg, and that of the medulla is only about 10-20mmHg [12]. This is primarily due to differential oxygen demand, dependent on tubular sodium reabsorption[13]. Mathematical models also suggest that luminal flow in the proximal tubule is an important determinant of tissue PO_2 in the cortex[14].

Tubule epithelial cells from S3 segment of the proximal tubule in the cortico-medullary junction perform ATP dependent active transport and utilize oxygen-dependent oxidative phosphorylation (OXPHOS) and beta-oxidation of fatty acids (FAO) for energy production, while other tubule components in the inner medulla rely on glycolysis for energy production[15]. Because of this disparity of blood flow, oxygen tension, and energy source in cortex and medulla, the kidney is susceptible to injury. However, there exists a strong renal adaptive mechanism to maintain constant PO_2 and normal tubular function and prevent cellular damage during altered oxygen supply[16]. At the tissue level, an increase in RBF or oxygen delivery is largely compensated by elevated oxygen consumption and partly by arteriovenous shunting[17]. At the cellular level, hypoxia and hypoxia-inducible factor (HIF) signalling induce

renal peritubular fibroblasts to increase synthesis erythropoietin (EPO) and increase oxygen-carrying capacity[16].

Renal oxygen delivery in SI-AKI:

Sepsis results in microcirculation dysfunction[18]. Preclinical and clinical studies show divergent renal hemodynamics during SI-AKI. Sepsis associated increase in cardiac output can potentially increase or preserve RBF [19]. In humans with sepsis, RBF declines[20, 21]. Though Prowle *et al*[20] did not find an association between reduced RBF and renal function in septic patients, the early decline in RBF and oxygen metabolism were associated with increased tubular injury in the study by Skytte Larsson *et al*[21]. However, Tran *et al* [22] using blood oxygen level-dependent (BOLD) MRI did not observe overt changes in renal tissue oxygenation despite reduced oxygen delivery in mice during LPS-induced AKI. Wang *et al* [23] using pimonidazole (PIM)-protein adducts to assess renal tubular hypoxia, observed cortical hypoxia in a cecal ligation puncture model as early as 4 hours after surgery. The inconsistencies among these findings could largely be due to the complex pathogenesis of sepsis, as well as due to the variation in experimental approaches to assess renal oxygenation in different studies.

Metabolic reprogramming in the kidney during SI-AKI:

Another key event that contributes to SI-AKI is metabolic reprogramming. It was first identified in rapidly dividing cancer cells where a lack of oxygen supply switched their metabolism from aerobic respiration to anaerobic respiration, a phenomenon known as Warburg effect[24]. Warburg effect has been observed in many other cell types beside cancer cells. During sepsis, the metabolic reprogramming is biphasic, initial glucose-dependent anabolic phase followed by

OXPHOS and FAO dependent catabolic phase[25]. Activation of the innate immune system and reduced oxygen delivery to the proximal tubule cells induces HIF1α signalling and switches cells towards less efficient anaerobic glycolysis for ATP production[26] followed by activation of the adenosine monophosphate kinase (AMPK) pathway[27]. This switch to anaerobic glycolysis could serve multiple purposes: 1) by undergoing less efficient pathway of energy production, cells can still maintain ATP production to maintain basal cellular activity and avoid potential cell death[24], 2) reduced OXPHOS activity leads to less mitochondrial reactive oxygen species(ROS) production and less cellular damage[28], and 3) activation of pentose phosphate pathway leads to increased production of nicotinamide adenine dinucleotide(NAD+)[29], a potent ROS inhibitor that exerts protection during SI-AKI[9]. Paradoxically, the metabolic switch could also contribute to AKI pathology. It has been shown that improving OXPHOS early on during SI-AKI in mice results in improved outcome[30], so do the late activation of AMPK and Sirtuin-1[25]. Understanding these temporal changes in metabolism during SI-AKI could serve as the key to identifying metabolic targets.

Multi-parametric Photo-acoustic microscopy

Current techniques available for assessment of renal oxygenation employ either oxygen microelectrodes[31] (polarographic electrodes), optical probes(optodes), magnetic resonance imaging (MRI) techniques including blood oxygenation level-dependent MRI (BOLD-MRI)[22] and dynamic nuclear polarization MRI (DNP-MRI) with an oxygen-sensitive paramagnetic agent (OX63)[32], and phosphorescence lifetime imaging microscopy (PLIM) using phosphorescence tracer element[33]. While classical oxygen microelectrode and optode based techniques can

directly measure renal oxygenation in the precise location in the kidneys, these techniques cannot give a continuous measurement. MRI based techniques can provide continuous assessment of renal oxygenation but accurate quantification of the oxygenation is still a hindrance. PLIM can provide the status of hypoxia at molecular details, but the low penetration of intravital imaging allows measurement of changes only in the cortex. Besides, none of the currently existing techniques can provide simultaneous measurement of blood flow, oxygen saturation and oxygen extraction.

Multi-parametric PAM is a novel technique that allows intra-vital high-resolution, quantitative, and comprehensive characterization of hemodynamics and oxygen metabolism and associated microvascular pathology in animal models[34]. PAM enables concurrent imaging of blood perfusion, oxygenation and flow without the use of any tracers of fluorophore molecules. These parameters can then be used to derive other parameters including oxygen extraction fraction and metabolic rate of oxygen. By distinguishing the spectra of oxy- and deoxy-haemoglobin, repetitive pulsed dual-wavelength excitation provides details on oxygen concentration and saturation of hemoglobin at capillary levels allowing for dynamic monitoring of the metabolic rate of oxygen. This technique has been established and validated for in-vivo imaging in the study of brain vasculature[35]. A penetration depth of 200 μm allows measurement of hemodynamic changes in peritubular capillaries in the cortex of exteriorized kidneys of an anesthetized mouse. Repetitive measurements can be performed in the same animal over a prolonged period to accurately measure temporal changes after inducing AKI. We are currently employing this method to determine tissue oxygen delivery in the kidney during sepsis. PAM can also be used with other imaging techniques, for example, two-photon microscopy to

simultaneously assess metabolic and microcirculatory changes during heath and disease. Identification of these vital parameters including tissue oxygenation during AKI will permit a better understanding of mechanisms of AKI that will inform therapeutic interventions to improve outcomes of patients with AKI.

Figure Legend

Figure. Photoacoutic Microscopy. Photoacoustic microscopy is a hybrid of optics and ultrasound. In photoacoustic microscopy, usually, a focused laser pulse is directed into biological tissue (kidney). The tissue absorbs light and induces transient heating. The thermoelastic expansion of the tissue converts the heat into acoustic emission. A transducer outside of the tissue, can detect the acoustic wave and form an image. The conversion from optical absorption to acoustic emission has a few unique advantages. First, photoacoustic imaging is solely sensitive to optical absorption creating a very specific imaging contrast. Second, relying on focused acoustic detection photoacoustic microscopy can operate beyond the penetration of pure optical microscopy.

Table 1. Applicability and limitation of current techniques to assess renal hemodynamics and oxygenation

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