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Sepsis-Induced Renal Failure: Ischemia or Toxemia?*

Elliott D. Crouser, MD
Division of Pulmonary, Critical Care and Sleep Medicine
The Ohio State University Wexner Medical Center
Columbus, OH

Sepsis was recently redefined (i.e., Sepsis-3) as "life-threatening organ dysfunction caused by a dysregulated host response to infection" (1). This implies that host factors occur in response to severe infections, most notably inflammatory cell activation and hemodynamic instability, resulting in multiple organ failure. This definition is a departure from early experimental evidence by founding members of the Society of Critical Care Medicine, including Broder and Weil (2), showing a strong inverse correlation between blood lactic acid levels and survival in critically ill humans, implicating tissue ischemia as a primary mechanism of organ dysfunction and death. However, clinical trials designed to optimize hemodynamic variables, along with oxygen and nutrient delivery, have failed to improve the outcome or demonstrate a survival benefit in human sepsis (3). Despite the lack of evidence linking energy substrate delivery to organ failures during sepsis, many of the tenets of critical care treatment for sepsis, including hemodynamic resuscitation bundles, serum lactate, and tissue oxygen saturation monitoring, remain focused on optimizing tissue oxygen delivery in order to prevent ischemic damage.

It is possible that blood supply is not the solution and actually contributes to the pathophysiology of severe sepsis. Is it possible that the blood is literally "poisoned" in the context of sepsis and that organ injury results from toxemia? According to this hypothesis, organ damage could occur despite "adequate" tissue oxygenation, and researchers would have to redirect their focus on components of blood that cause organs to fail and how to eliminate or neutralize them.

A growing body of evidence indicates that circulating factors initiate cell and possibly organ dysfunction during sepsis. Nearly a quarter of a century after the seminal lactate mortality correlation observation of Broder and Weil (2), Tracey et al (4) reported in the journal "Nature" that pretreatment of baboons with a tumor necrosis factor (TNF)–α blocking antibody ameliorated experimental *Escherichia coli* induced septic

*See also p. e318.

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shock and associated mortality. Kumar et al (5) further identified TNF-α as a circulating mediator of acute sepsis–induced myocardial dysfunction. Additional insight into the mechanisms of TNF-α–induced organ failures was provided by Tran et al (6), wherein TNF-α was linked to impaired kidney oxygen consumption despite normal blood oxygen tensions in rodents. Reduced tissue oxygen utilization in this model was shown to result in suppression of mitochondrial gene expression, which was reversed by agonists of peroxisome proliferator-activated receptor y coactivator-1a, a potent promoter of mitochondrial biogenesis. Under other experimental conditions, TNF-α was shown to induce acute mitochondrial energy failure due to the induction of toxic mitochondrial reactive oxygen species (ROS) (7–9). Thus, circulating factors, particularly TNF-α, are shown to promote the rapid onset of cytopathic changes associated with impaired mitochondrial energy production and increased mitochondrial ROS production.

Based upon these and other preclinical observations, neutralizing circulating sepsis mediators, particularly TNF-α, were considered plausible therapeutic targets in human sepsis. Unfortunately, a primary role for TNF-α as a cause of organ failures and related mortality in human sepsis was not supported by the results of a large, randomized, double-blind, placebo-controlled clinical trial that failed to show a survival benefit (10). However, the timing of the intervention is critical. Peak TNF-α levels are reached in serum within hours of the onset of septic shock in humans or animal models, after which they quickly normalize (11, 12). This rapid dissipation of TNF-α could explain why blood samples randomly obtained hours or days after the onset of severe sepsis fail to induce cytopathic mitochondrial changes (e.g., in rat muscle) (13), which is in contrast to mitochondrial suppression induced by septic serum in which elevated levels of TNF-α are confirmed (14). Given the narrow therapeutic window for TNF-α and presumably for other proximal proinflammatory mediators in sepsis, it is logical to consider downstream targets. Blocking downstream effects of circulating sepsis mediators, such as mitochondrial ROS production, would be an alternative approach to ameliorating cytopathic injury during sepsis.

In this issue of *Critical Care Medicine*, Arulkumaran et al (15) sought to determine mechanisms explaining renal functional impairment despite preserved hemodynamics and histology in sepsis-induced acute kidney injury. More specifically, they sought to establish the contribution of circulating factors associated with sepsis on renal tubular mitochondrial dysfunction, as has been previously reported in animal models of sepsis (16, 17). To this end, a novel, nonlethal rodent fecal peritonitis model using implanted standardized human fecal pellets was employed. The model features modest, although significant lactate elevation (mean of 1.7 vs 1.3 mmol/L in sepsis vs controls), with no overall change in systemic or renal oxygen metabolism. The effect of septic serum was further assessed in live kidney slices obtained from the same rodent population. A novel multiphoton confocal imaging technique was used to evaluate live kidney slices exposed to septic serum, allowing for dynamic monitoring of nicotinamide adenine dinucleotide (NADH, the primary source of electrons for mitochondrial electron transport), mitochondrial membrane potential (MMP, providing energy for adenosine triphosphate (ATP) production), and ROS (a potentially toxic byproduct of ATP formation). Despite unchanged oxygen metabolism variables, sepsis was associated with impaired renal function (elevated creatinine), and exposure to septic serum in vitro caused reduced NADH and MMP and increased renal ROS production. The protective effect conferred by 4-hydroxy-2,2,6,6-tetramethylpiperidine-N-oxyl, a free radical scavenger capable of dismutating superoxide radical and reducing superoxide production by other mechanisms (18), infers a direct mechanistic link between sepsis-induced ROS and renal tubular epithelial dysfunction. This interpretation was not apparently supported by measures of urine isoprostanes, a stable metabolite of oxidized fatty acids, which were not significantly elevated in septic animals. However, urine isoprostane levels may underestimate renal tissue oxidant stress due to dilution of kidney-derived isoprostanes by those formed in other systemic organs and tissues (i.e., derived from filtered blood). Furthermore, tissue oxidant stress in the kidneys is likely to be mitigated by the observed overexpression of mitochondrial uncoupling protein (UCP)–2 in renal tissues, which effectively reduces formation of ROS during aerobic (mitochondrial) respiration. The downside of UCP2 overexpression being the diversion of energy substrates needed for mitochondrial electron transport (e.g., consumption of NADH) away from ATP formation (i.e., a reduction in the energy gradient across the mitochondrial inner membrane that is required for ATP formation, as reflected by lower MMP) (19). As such, UCP2 overexpression presumably reduces ROS formation at the expense of impaired cell and organ function, as reflected by acute kidney injury (AKI) in the study by Arulkumaran et al (15).

The study by Arulkumaran et al (15) stands out as the first to show that sepsis-induced AKI is promoted in the absence of altered tissue oxygen (energy substrate) supply, as reflected by unchanged regional tissue oxygen metabolism, while simultaneously demonstrating that septic serum is sufficient to promote renal tubular dysfunction, as reflected by altered mitochondrial bioenergetics variables and impaired glomerular filtration. Thus, the study unequivocally incriminates serum factors, the identity of which remains to be determined, as the cause of ROS induction and related renal tubular dysfunction during sepsis.

Although the results are compelling, there are a number of study limitations and related unanswered questions. First and foremost, relatively young rodents are not equivalent to the average septic human, who are typically over 60 years old and frequently have comorbid conditions. This common limitation of animal research undoubtedly contributes to the failure

of animal model observations to reliably translate to human sepsis. The model is also influenced by the nature of the sepsis source, a human fecal pellet of variable composition, and the composition of septic serum, which is known to change over time (11, 12). Finally, this nonlethal model does not promote hemodynamic instability, which represents a potential "second hit" that is expected to exacerbate AKI in the setting of severe sepsis or septic shock in humans.

The study by Arulkumaran et al (15) confirms that sepsis-induced AKI during mild forms of sepsis is primarily a consequence of "toxemia" rather than "ischemia" with impaired blood flow. Given the current emphasis of ICU care on monitoring and optimization of blood flow, the study by Arulkumaran et al (15) emphasizes the need to expand current sepsis treatment paradigms to better understand basic mechanisms of sepsis-induced organ failures. Future ICUs will likely monitor sepsis mediators and related cytopathic events to determine the phase of sepsis and to provide the most appropriate, personalized therapies.

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