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ADVANCES IN SEPSIS

Commentary and analysis on advances in the understanding and treatment of sepsis

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Dear Colleague,

Welcome once again to *Advances in Sepsis*.

In the first article of this issue, Drs Elliott Crouser and Stephen P Hoffmann (The Ohio State University, Columbus, OH, USA) review the potential role of altered mitochondrial function as a major cause of the metabolic alterations seen in severe sepsis. The authors discuss the evidence to suggest that mitochondrial damage contributes to organ failures, and describe mechanisms of mitochondrial dysfunction during sepsis, in particular, the effects of oxidants on mitochondrial function. Furthermore, they discuss a potential role in sepsis of a specific marker of mitochondrial damage.

Dr Undurti Das (UND Life Sciences, Shaker Heights, OH, USA) assesses the potential use of ethyl pyruvate as a therapy in sepsis. Dr Das discusses the anti-inflammatory actions of ethyl pyruvate, its activity as a free radical scavenger, and *in vivo* studies demonstrating beneficial anti-inflammatory effects. Lastly, the involvement of pyruvate in glucose metabolism and its effects on insulin production is assessed.

Septic patients may develop hyperglycemia, and one of the recommendations of the SSC is to maintain adequate glycemic control. In the Surviving Sepsis section of this issue, Drs Armelle Mathonnet and Alain Cariou (Cochin Hospital, Paris 5 René Descartes University, Paris, France) evaluate studies assessing the importance of strict regulation of glycemia in critically ill patients. The authors suggest that a system should be developed for continuous glycemic monitoring prior to considering intensive insulin therapy for all critically ill patients, and that this could reduce the occurrence of adverse effects of insulin treatment.

This is followed by our regular SSC update. In this issue, the SSC Steering Committee Members respond to the article from Peter Eichacker and colleagues that was published towards the end of last year in the *New England Journal of Medicine*, which took the position that the SSC and the associated guidelines have been influenced by Eli Lilly.

As in all issues, the Clinical Reviews provide concise and critical analyses of the latest and most important sepsis literature, placing recent developments in a clinical context.

We would like to thank all those readers who have taken time to provide feedback on the articles presented in *Advances in Sepsis*. We are pleased that the comments have been overwhelmingly positive and that the journal continues to be regarded as a useful resource by clinicians working in this fast-developing field.

We hope that you will continue to submit articles, ideas, and suggestions for future issues.

Benoît Vallet and Mitchell M Levy

Editors-in-Chief

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Aims and Scope
Advances in Sepsis is designed to bring a critical analysis of the recent world sepsis literature, written by clinicians, for clinicians, to an international, multidisciplinary audience. Our mission is to promote better understanding and treatment of severe sepsis and septic shock across the global healthcare system by providing an active forum for the discussion of clinical and healthcare policy issues.
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LEADING ARTICLE

Mitochondrial Mechanisms of Organ Dysfunction During Sepsis

Stephen P Hoffmann[1,2] and Elliott D Crouser[1,2,3]

[1]Division of Pulmonary, Sleep and Critical Care Medicine, The Ohio State University Medical Center, [2]Dorothy M Davis Heart and Lung Research Institute and [3]Institute of Mitochondrial Biology, The Ohio State University, Columbus, OH, USA

Acute bacterial and fungal infections that are associated with organ failures (sepsis) account for hundreds of thousands of deaths worldwide each year, yet the primary cause of sepsis-associated organ failures remains elusive. Inadequate tissue perfusion (shock) is capable of undermining organ function; however, organ failures and death frequently occur in septic patients even in the absence of shock. A growing number of animal models and some human studies indicate that cell metabolism is fundamentally altered during sepsis. This review considers the evidence supporting damage to mitochondria, the primary energy providers of the cell, as a major determinant of sepsis-induced organ failures.
Adv Sepsis 2007;**6**(1):2–9.

Sepsis is the leading cause of death in the intensive care unit (ICU), and most of these deaths are attributed to the multiple organ dysfunction syndrome (MODS). Uncontrolled systemic inflammation and inadequate tissue perfusion (shock) are the putative mechanisms through which sepsis induces organ failures. Evidence for these mechanisms comes from animal and human studies that demonstrate improved survival when anti-inflammatory agents or reversal of shock are rendered in the early phases of sepsis. However, inhibition of inflammatory responses, or measures designed to optimize tissue perfusion are shown to have little or no benefit in the context of established sepsis (i.e. sepsis associated with one or more organ failures). Moreover, a growing body of evidence indicates that sepsis-induced organ failures are associated with fundamental alterations of metabolism at the cell and tissue level and that tissue hypoxia does not play a central role in this process. This review will focus on the possible role of altered mitochondrial function as a major cause of the metabolic alterations attendant to severe sepsis.

The controversy surrounding mitochondrial mechanisms of organ failure

Over 40 years ago, Broder and Weil observed a strong correlation between elevated blood lactate levels and increased mortality rates in critically ill patients [1]. Increased blood lactate was interpreted to reflect tissue hypoxia (anaerobic metabolism), which has emerged as the putative cause of organ failures in critically ill patients, including those with severe sepsis [2]. Indeed, most of the monitoring equipment presently employed for the management of critically ill patients, including pulmonary artery catheters, oxygen saturation probes, and blood pressure cuffs, are designed to detect inadequate tissue oxygen delivery.

Recent reports provide convincing evidence that shock contributes significantly to the risk of mortality during sepsis; however, the mortality rate exceeds 30% even when shock is preemptively treated, suggesting that inadequate tissue oxygen delivery, as reflected by conventional hemodynamic parameters and circulating lactate levels, is not the sole cause of organ dysfunction and attendant mortality [3]. However, it should be noted that covert tissue hypoxia may be missed when conventional clinical parameters are employed for the detection of blood flow [4]. Even so, tissue oxygen levels are shown to be normal or even elevated in adequately resuscitated sepsis [5–7], and attempts to increase systemic oxygen delivery in established severe sepsis provide no survival benefit [8–9]. Levy et al. provide evidence that elevated lactate concentrations during sepsis are reflective of inherent changes in glucose metabolism, which are unrelated to tissue hypoxia [10]. Furthermore, elevated pyruvate levels in the setting of hyperlactatemia, which occurs in severe sepsis, is inconsistent with the tissue hypoxia paradigm, instead implying dysregulation of metabolic pathways that are operating distal to glycolytic pathways [11].

Address for correspondence: Elliott D Crouser, The Ohio State University Medical Center, 201F DHLRI, 473 West 12th Avenue, Columbus, OH 43210-1252, USA. Email: elliott.crouser@osumc.edu

Research in animal models supports the notion that fundamental changes in cellular metabolism, as opposed to inadequate supply of energy substrates, is the cause of altered metabolism during sepsis. Over 30 years ago, Mela and colleagues demonstrated mitochondrial damage and dysfunction in response to direct exposure to bacterial endotoxin [12] and in mitochondria that were isolated from the tissues of septic animals [13]. However, subsequent studies variably reported unchanged or even supernormal mitochondrial function in different sepsis models [14,15]. In some cases, the findings are explained by technical limitations, including highly selective mitochondrial isolation techniques, which tend to exclude the most damaged mitochondria from analysis [15]. Other studies failed to preserve mitochondrial integrity during the isolation process, as reflected by profound dysfunction of mitochondria in the non-septic control groups [14]. Other important variables that influence the experimental results include the model of sepsis used, including the site of infection, the toxicity of the organisms or bacterial components used to inoculate the animal, the use of fluid resuscitation and antibiotics, and the species of animal employed (see discussion by Singer and Brealey. [16]). In the final analysis, the optimal model should reflect the human condition.

Within the past decade, a body of research has provided convincing evidence of mitochondrial pathology in vital organs in animal models of sepsis. Several groups independently observed tissue acidosis – traditionally viewed as a sign of tissue hypoxia – in the setting of elevated tissue oxygenation, indicating abnormal tissue oxygen utilization in septic animals [7,17]. These findings were substantiated by Piantadosi et al. who observed reduced oxygen consumption in liver tissue extracted from endotoxin-treated animals [15]. Using a feline endotoxemia model, the current authors' laboratory reported a correlation between mitochondrial damage and altered oxygen utilization in an intact, perfused organ (ileum), and further demonstrated that mitochondrial damage was unrelated to tissue hypoperfusion or hypoxia [18,19]. Thus, abnormal mitochondrial function apparently can develop independently of tissue hypoperfusion (ischemia) in the context of sepsis.

Cellular metabolism is difficult to assess in real time in human tissues, particularly in the setting of acute illness; however, indirect evidence of mitochondrial damage and dysfunction in septic humans is provided by several investigations. In 1979, Trump et al. reported the results of immediate autopsies performed on patients who died of various critical illnesses [20]. In one dramatic case the patient died within 24 h of the onset of overwhelming infection related to bowel perforation. Dramatic ultrastructural changes and impaired respiratory function were documented in mitochondria that were rapidly recovered from various vital organs in multiple organ systems [20]. Likewise, Van den Berghe et al. recently documented ultrastructural mitochondrial damage in a representative vital organ (liver) in a cohort of sepsis non-survivors [21]. Mitochondrial damage could explain decreased adenosine triphosphate levels and reduced mitochondrial complex I activity in muscle tissue of patients who eventually died of sepsis [22]. A recent study by Fredriksson et al. reported that mitochondrial content was reduced by more than 50% in diaphragm and leg muscle biopsies obtained from patients with sepsis-induced MODS relative to non-septic controls [23]. Thus, the human data corroborate the findings of animal models and support a role for mitochondrial damage and depletion in the context of sepsis-induced MODS.

Aside from oxygen utilization, the metabolism of essentially all metabolic substrates is altered during severe sepsis, and mitochondria appear to contribute significantly to this phenomenon. Increased protein catabolism, impaired β-oxidation of long-chain fatty acids, and inhibition of ketogenesis are well-documented complications of severe sepsis [24,25]. Using a proteomic technique that makes no *a priori* assumptions regarding which, if any, mitochondrial pathways might be affected (i.e. an unsupervised analysis), protein expression changes in liver mitochondria in response to systemic administration of *Escherichia coli* endotoxin supports a likely role of mitochondria in many of the metabolic alterations that occur during sepsis. With respect to protein metabolism, the expression of rate-controlling enzymes of the urea cycle was shown to be significantly increased, which has implications for protein catabolism and excessive nitric oxide production. Conversely, the expression of acyl-coenzyme A (CoA) dehydrogenase and hydroxymethylglutaryl (HMG)-CoA synthase, key enzymes regulating the β-oxidation of lipids and ketogenesis, respectively, were significantly reduced [26]. Thus, adaptations of mitochondria to conditions of sepsis at the level of gene expression could explain many of the metabolic alterations that are observed in septic patients.

Mitochondria and cell death

The implications of acute mitochondrial damage extend beyond metabolic functions and during the extremes of cellular stress, mitochondria play an important role in "life-and-death" decisions. Cell damage of any cause that is associated with super-physiological accumulation of reactive oxygen species (ROS), calcium, or the induction of certain cell signaling pathways (e.g. by tumor necrosis factor-α [TNF-α]) can trigger highly regulated "mitochondrial death signals". The critical step in this process is the opening of large conductance channels called mitochondrial

permeability pores, which span the inner mitochondrial membrane. This phenomenon, referred to as the mitochondrial permeability transition (MPT), results in high-amplitude swelling of the mitochondrial matrix, rupture of outer mitochondrial membranes, and consequent release of various factors that initiate programmed cell death (apoptosis). The apoptotic cascade that follows requires energy (ATP) and new protein synthesis, culminating in the formation of neatly packaged, partially degraded "apoptotic bodies", which are then removed by regional macrophages [27]. Under certain conditions and in specific cell types, the release of mitochondrial apoptotic factors appears to occur through the direct movement of pro-apoptotic factors across the outer mitochondrial membrane and does not require the MPT; this process is referred to as mitochondrial outer membrane permeabilization (MOMP) [28]. Finally, under conditions of extreme and overwhelming cell stress, such as occurs after prolonged tissue ischemia and subsequent reperfusion, cellular ATP levels acutely decline and the integrity of all cell membranes is compromised, resulting in widespread MPT-induced mitochondrial swelling and cell lysis (necrosis). This is an unfavorable outcome for the host because, unlike apoptosis, necrosis potently induces regional and systemic inflammation [29].

In contrast to conditions of acute sepsis complicated by refractory shock, which results in acute and widespread cell necrosis [20], cell death appears to play a limited role during the terminal phase of severe sepsis. Expecting to find widespread cell death in vital organs in patients who had recently died of sepsis, Hotchkiss and colleagues observed increased apoptotic cell death that was localized to the gut epithelium, spleen, and circulating lymphocytes only [30]. Mitochondrial apoptotic pathways were shown to play a critical role in the death of immune cells [31]. The latter presumably promotes "immune paralysis" and predisposition to nosocomial infection in the late phase of sepsis; however, it is clear from these investigations that massive cell death does not explain sepsis-induced vital organ failures, which are the ultimate cause of death in most septic patients.

Evidence that mitochondrial damage contributes to organ failures

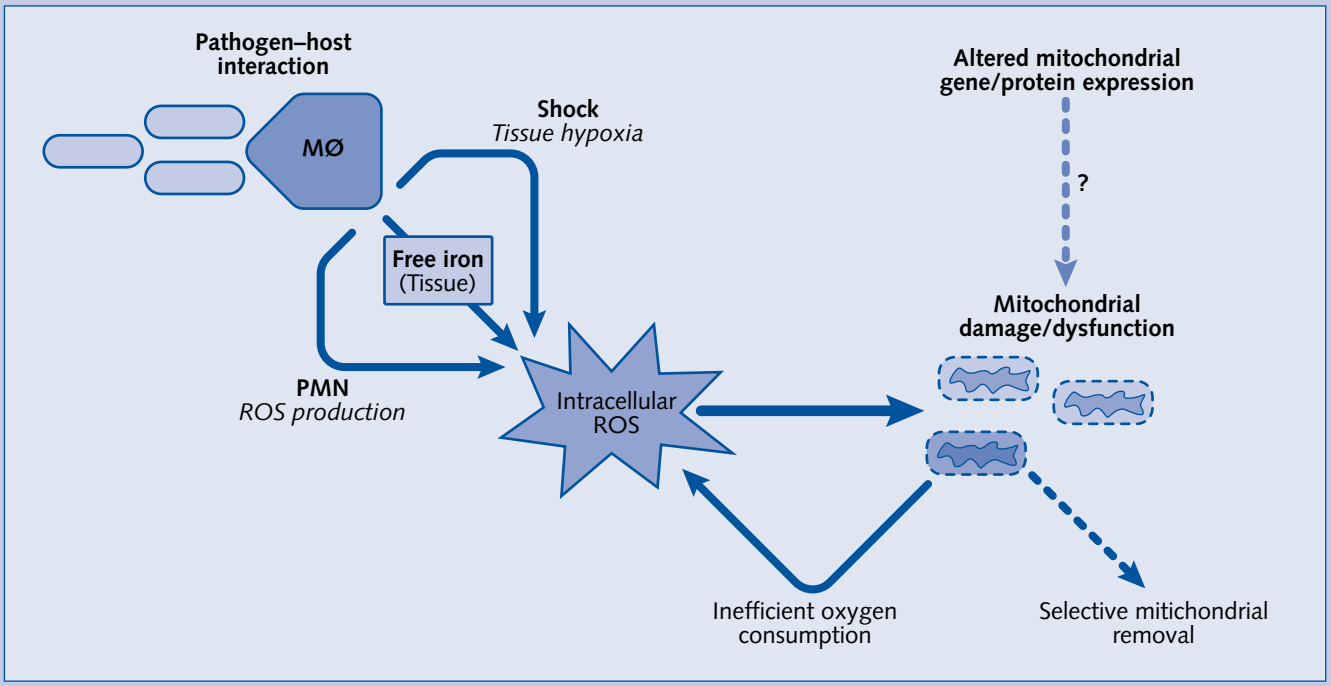
As it is difficult to isolate mitochondrial functions from other metabolic pathways, definitive proof that changes in mitochondrial function contribute directly to impaired cell and organ function during sepsis is not currently available. In metabolically active tissues, such as the brain and working muscle, mitochondrial ATP production is essential for normal function. It follows that a critical threshold of mitochondrial dysfunction would result in organ failure, which explains why metabolically active organs, such as muscle, kidney, and brain, are susceptible to failure during sepsis. Recent studies performed in relevant animal models provide compelling support for a causal relationship between altered mitochondrial function and vital organ failure in the context of sepsis. In a highly lethal model of bacterial septic shock that is associated with impaired cardiac contractile function, Watts and colleagues observed dramatic depletion of mitochondrial populations and attendant mitochondrial dysfunction in the heart [32]. However, evidence for a statistical correlation between mitochondrial respiratory dysfunction and abnormal cardiac contractility and relaxation times in a sepsis model, was recently reported by two independent studies [33,34]. Other laboratories have confirmed that toxic doses of sepsis mediators (lipopolysaccharide [LPS]) potently inhibit contractile and mitochondrial functions in diaphragm and cardiac muscle [35,36]. By contrast, in a fluid resuscitated dog model, Solomon et al. failed to observe a change in tissue ADP levels in association with impaired cardiac contractility. However, in contrast to untreated animals, it is interesting to note that septic dogs failed to further increase their myocardial performance (work) in response to catecholamines [37]. Thus, it would appear that septic animals are operating at or near their maximal cardiac performance at baseline, perhaps due to limited mitochondrial ATP production. In support of this notion, there is known to be a dynamic interplay between the transfer of high energy phosphates, including ADP and ATP, and cardiac contractility [38,39], which presumably matches energy consumption with energy supply to avoid ATP depletion and associated cell death [40].

The impact of mitochondrial dysfunction in adult human populations may be underestimated in experiments conducted in young and healthy animals, which is the standard approach. Considering that gradual loss of mitochondrial functional reserve and associated changes in metabolism are common manifestations of aging [41], and that elderly patients are susceptible to multiple organ failure [42], it is interesting to speculate that elderly patients are particularly sensitive to sepsis-induced mitochondrial dysfunction. Thus, appropriate experimental models of human sepsis should consider the influence of advanced age.

Mechanisms of mitochondrial dysfunction during sepsis

Sustained shock or ischemia/reperfusion is sufficient to cause mitochondrial damage and dysfunction. However, tissue oxygenation is found to be normal or even supernormal in patients with severe sepsis, and mitochondrial damage is observed despite adequate tissue oxygenation under conditions modeling sepsis [18]. Alternatively, direct and

Figure 1. Mechanisms of mitochondrial damage during sepsis. Variables relating to the characteristics of the pathogen–host interaction, including the virulence of the microorganism and the quality of the host immune response, dictate the duration and magnitude of the sepsis syndrome. In severe cases, systemic activation of immune cells, particularly neutrophils, and tissue hypoxia (shock) contribute to the formation of ROS. ROS, in turn, contribute to the release of intracellular iron and mitochondrial damage, events that further contribute to ROS formation. This cycle is ultimately broken by the removal and replacement of damaged mitochondria and resolution of systemic inflammation. The contribution of altered gene and protein expression towards the development of mitochondrial dysfunction in vital organs during sepsis remains to be determined.



MØ: Macrophage; PMN: polymorphonuclear leukocyte; ROS: reactive oxidant species.

indirect cytotoxic actions of pro-inflammatory cytokines may be responsible.

TNF-α is a cytokine with pleiotropic actions, including the induction of mitochondrial "death pathways". Blood and tissue TNF-α levels are dramatically elevated in the early phase of sepsis [43], and TNF-α has the capacity to directly confer damage to mitochondria [44]. Recognition of TNF-α by TNF receptor-1 (TNFR-1) results in the activation of specific caspases and intracellular release of ceramide, which are agents that promote the MPT [45]. The mitochondrial toxicity of TNF-α is attenuated by cyclosporin A, a pharmacological inhibitor of mitochondrial permeability pore opening [46], and is shown to confer mitochondrial protection in vital organs during Gram-negative endotoxemia [33,47,48]. Likewise, overexpression of endogenous inhibitors of the MPT (e.g. Bcl-2 proteins) protects against mitochondria-mediated cell death in mononuclear immune cell lines and in the intestinal epithelium [49], improves vital organ function [34], and significantly reduces the mortality rate in murine sepsis models [34,49].

Sepsis promotes increased oxidant stress in vital organs, and mitochondria serve as both a source and target of ROS (Fig. 1). Mitochondria are the primary source of ROS in healthy cells, and high levels of antioxidants are concentrated in the mitochondrial matrix in order to quench these potentially harmful byproducts of oxidative phosphorylation. During sepsis, mitochondria become overproducers of ROS [50,51] and there is a shift in the expression of critical antioxidant enzymes, including increased expression of superoxide dysmutase relative to catalase, which together promote the overproduction of ROS, and subsequent oxidative damage to the cell [52,53]. Mitochondria are susceptible to oxidative stress during sepsis, and impaired mitochondrial function is an expected consequence [33,36,54]. Although the uncoupling of mitochondrial respiration from ATP formation observed during sepsis undermines the capacity of the cell to perform work, it may confer protection by reducing the rate of ROS production [33].

The oxidant stress paradigm is supported by recent investigations showing improved outcomes with antioxidant treatments. Very recently, Supinski et al. demonstrated potent protection against mitochondrial damage in the hearts of endotoxemic rats that were pretreated with a cell-permeable

mimic of an endogenous antioxidant enzyme (superoxide dysmutase) [36]. Likewise, Ritter et al. reported protective effects of combination therapy with an iron chelator (desferoxamine) and glutathione precursor (*N*-acetylcysteine) [52]. The benefits of iron chelation are based on the observation that sepsis promotes the liberation of intracellular iron, which, in turn, reacts with oxygen to promote highly toxic hydroxyl radical formation (i.e., Fenton reaction) [55]. However, one should be careful not to categorize all oxidants as toxic. For example, nitric oxide (NO) reacts readily with cysteine thiols to modify and presumably regulate the activities of various mitochondrial enzymes under physiologically relevant conditions [56]. NO reacts immediately with highly toxic superoxide anions to form peroxinitrite, which contributes to protein nitration. Nitration and denitration of mitochondrial proteins is shown to occur rapidly in normally respiring mitochondria [57], suggesting that protein nitration events are not pathological, at least in healthy mitochondria. Supernormal concentrations of NO, such as are observed during sepsis, are capable of inhibiting mitochondrial respiration [58] while suppressing energy consuming processes (e.g. muscle contractility [59]), which may be essential for reducing metabolic demands in the face of acute cell injury. In this context, it would appear that suppression of NO formation in the heart results in improved cardiac and mitochondrial performance, but results in irreversible oxidative protein damage (carbonylation) in the heart [60]. This might explain why nonspecific inhibition of NO synthase enzymes, the primary source of NO during sepsis, improves hemodynamic indices (e.g. mean arterial pressure), but increases sepsis mortality rate in humans [61]. Thus, it is reasonable to speculate that sepsis-induced organ failures serve a protective role by reducing cell work under conditions of oxidative cell injury, as suggested by Singer et al. [62].

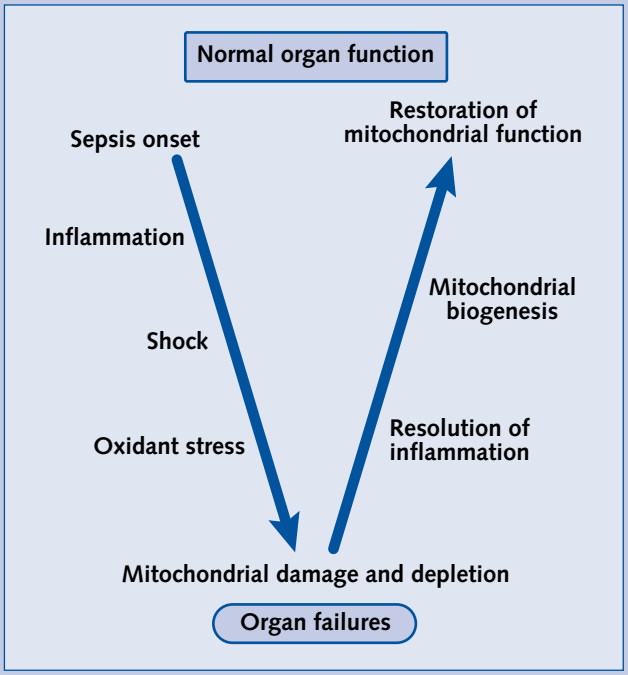
Sepsis-induced mitochondrial damage promotes increased ROS production, and the process is expected to be self-perpetuating. Fortunately, mechanisms are in place to target damaged or senescent mitochondria for removal from the cell, thereby interrupting the cycle of mitochondrial ROS overproduction and progressive mitochondrial dysfunction. One manifestation of mitochondrial damage caused by oxidant stress, is a change in electrochemical gradient ($\Delta\Psi$) that forms across the inner mitochondrial membrane in response to electron transport and consequent hydrogen ion efflux from the mitochondrial matrix. Dissolution of $\Delta\Psi$ may occur by a number of mechanisms, including opening of mitochondrial permeability pores and increased electron "leak" during electron transport. Regardless of the cause, a sustained reduction in $\Delta\Psi$ activates specific calcium-independent phospholipase enzymes that begin to degrade mitochondrial membranes [63]. Recent studies suggest that these mitochondria are effectively taken "offline" in that they no longer respire [54], and presumably no longer produce excessive amounts of ROS. These mitochondria are subsequently targeted for lysosomal degradation (autophagy) [54,64]. The resultant depletion of mitochondria is transient providing that simultaneous mitochondrial biogenesis occurs, such as is demonstrated in models of sepsis [54,65]. Ultimately, this process leads to the removal of oxidatively damaged and dysfunctional mitochondria (Fig. 1) [54].

In addition to damage and depletion, recent investigations indicate that mitochondrial functions are under genetic control during sepsis. Human volunteers were systemically treated with a low-dose bolus of Gram-negative bacterial endotoxin and peripheral blood monocytes (PBMC) were analyzed for gene expression changes at various timepoints thereafter. Initially, as expected, pro-inflammatory gene networks were activated in response to LPS. However, gene expression in PBMC at later timepoints was characterized by downregulation of gene networks regulating metabolic activities, particularly mitochondrial functions [66]. It is interesting to speculate that suppression of PBMC metabolic pathways may be one mechanism by which the intense immune response that occurs upon initial exposure to infection is modulated to prevent secondary injury to the host. Alternatively, the downregulation of metabolic pathways in immune cells may contribute to the "immune paralysis" that commonly occurs in the late phase of sepsis, which predisposes the patient to secondary, life-threatening hospital-acquired infections [67]. These findings, together with the observed decreases in ATP levels and mitochondrial complex I activity in muscle in sepsis non-survivors [22], suggest that bioenergetic failure due to altered mitochondrial function is a global problem affecting diverse cell and tissue types and potentially contributing to vital organ failures in humans (Fig. 2).

Mitochondrial biomarkers

The functional status of mitochondria in vital organs is difficult to measure *in vivo*, and the equipment required to do so (e.g. nuclear magnetic resonance imaging), is not readily applied to the clinical setting. However, specific biomarkers of mitochondrial damage are emerging. In an effort to identify novel sepsis biomarkers, Struck and colleagues performed an unsupervised analysis of total protein expression in blood samples of septic humans [68]. They identified a mitochondrial protein, carbamoyl phosphate synthetase-1 (CPS-1), which is primarily derived from liver mitochondria. Recently, CPS-1 was shown to reflect mitochondrial damage and depletion in the context of peritonitis in a mouse model. Notably, CPS-1 release occurs earlier than other liver enzymes [54]. The specificity of CPS-1

Figure 2. Proposed relationships between organ failures and mitochondrial events during severe sepsis.



as a sepsis biomarker is undetermined, but the authors predict that circulating CPS-1 levels would be elevated in the context of various forms of liver toxicity. Nonetheless, CPS-1 represents the first available biomarker of mitochondrial damage in a vital organ and may prove to be a useful marker of liver toxicity in various clinical settings, including sepsis.

Organ failures: a cytoprotective mechanism?

Beyond the limitations of cellular energy production caused by mitochondrial damage and depletion, there is a further strain on cellular metabolism attendant to repairing the damage itself. For instance, DNA damage is a common manifestation of sepsis and DNA repair is costly in terms of energy demands on the cell. DNA repair requires the activation of poly(ADP-ribose) polymerase (PARP), a process that consumes both nicotinamide adenine dinucleotide (NAD), which is a vital substrate for mitochondrial ATP synthesis, and ATP itself. Indeed, excessive activity of PARP-mediated DNA repair can severely deplete cellular ATP levels resulting in necrotic cell death, especially in cells relying on glycolytic substrates for mitochondrial ATP production [69]. It follows that reduced energy consumption in the form of cell and organ "work", would help to maintain nominal ATP stores in the face of impaired mitochondrial function and energy-dependent cell repair processes, in the context of sepsis-induced organ injury. Thus, suppression of cell and organ metabolism, such as that observed in the hearts [70] and livers [71] of septic animals, may serve to maintain cell viability in the context of decreased mitochondrial energy production and increased metabolic demands attendant to cell repair. To the extent that reduced cellular metabolism favors cell survival and eventual recovery, it follows that inhibition of cellular metabolism in the context of sepsis is genetically regulated, as indicated by recent studies demonstrating suppression of mitochondrial gene expression in diaphragm muscle [72] and in circulating monocytes [66].

Conclusion

The body of scientific evidence indicates that cell metabolism is abnormal in vital organs in the setting of severe sepsis. Mitochondria participate in numerous critical metabolic pathways, including the breakdown of lipids, glucose, and proteins for the purpose of high-energy phosphate production and other vital functions. The cumulative effects of oxidant stress, attendant mitochondrial damage, and altered expression of genes and proteins regulating mitochondrial metabolic pathways, results in compromised cell function or even cell death. As such, sepsis-associated limitations of mitochondrial performance almost certainly contribute to vital organ dysfunction and would explain the ineffectiveness of augmenting blood flow (increased energy substrate delivery) in patients with sepsis and established organ failures [8,9].

Fortunately, damaged mitochondria are efficiently removed and replaced, at least in otherwise healthy young animals [54]. Additional research is needed to fully understand these processes and to answer several critical questions. For instance, how are damaged mitochondria targeted for removal, and what are the key regulators of mitochondrial repletion? Is the restoration of normally functioning mitochondrial populations required for recovery of vital organ function? Most importantly, can therapies be devised to manipulate mitochondrial function in the context of critical illness to prevent or restore cell and organ function? In this context, novel antioxidant therapies [52,73] and measures designed to interrupt mitochondrial death pathways [34,49,74] are being tested in animals with encouraging results, but none of these approaches are currently employed during human sepsis. Moreover, the beneficial effects of insulin in terms of preserving mitochondria populations in vital organs [17] and preventing organ failures in critically ill patients [75] may relate to insulin's known capacity to increase mitochondrial biogenesis and thereby improve glucose tolerance [76]. Thus, there is hope that further advances in our understanding of the various roles played by mitochondria in the pathogenesis of disease will contribute to the development of more effective therapies for patients with sepsis and other forms of critical illness.

Disclosures

The authors have no relevant financial interests to disclose.

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LEADING ARTICLE

Ethyl Pyruvate and Sepsis

Undurti N Das

UND Life Sciences, Shaker Heights, OH, USA

Pyruvic acid is an effective scavenger of reactive oxygen species (ROS). Ethyl pyruvate (EP), a stable analogue of pyruvate, has potent anti-inflammatory actions and is of benefit in animal models of sepsis and septic shock. EP inhibits tumor necrosis factor-α (TNF-α) and high mobility group box-1 (HMGB1) production, and suppresses lipopolysaccharide-induced nuclear factor-κB (NF-κB) activation. EP also decreases messenger RNA (mRNA) expression of cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), and interleukin-6 (IL-6) in the liver, ileal mucosa, and colonic mucosa in animal models of endotoxemia. Sepsis and septic shock are characterized by increased production of free radicals, TNF-α, IL-6, and HMGB1, and activation of COX-2 and iNOS. As EP can suppress these abnormal biochemical and immunological events, it is suggested that EP may be useful in the treatment of the critically ill and those with sepsis/septic shock, either alone or in combination with other drugs. *Adv Sepsis* 2007;**6**(1):10–5.

Sepsis is caused by a systemic inflammatory response to infection or noninfectious disorders, and mortality in sepsis is a result of multiple organ dysfunction. The 1992 American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) Consensus Conference introduced the term "systemic inflammatory response syndrome" (SIRS) as a reference for the complex findings that result from a systemic activation of the innate immune response, which is triggered by localized or generalized infection, trauma, thermal injury, or sterile inflammatory processes such as acute pancreatitis [1]. A diagnosis of SIRS is considered when patients present with more than one of the following clinical findings:

- Body temperature >38°C or <36°C.
- Heart rate >90 beats/min.
- Hyperventilation, as evidenced by a respiratory rate of >20 breaths/min or a partial pressure of carbon dioxide of <32 mmHg.
- White cell count of >12 000 cells/μL or <4000 cells/μL.

The SIRS concept is now globally adopted and followed by many clinicians. Sepsis is defined as SIRS plus infection, while severe sepsis is defined as sepsis associated with organ dysfunction, hypoperfusion, or hypotension, and septic shock is defined as sepsis with arterial hypotension, despite adequate fluid resuscitation. The 2001 SCCM/European Society of Intensive Care Medicine/ACCP/American Thoracic Society/Surgical Infection Society International Sepsis Definitions Conference noted that these definitions of sepsis, severe sepsis, and septic shock are robust and there is no necessity to change the criteria of these definitions; however, it was recognized and agreed that the signs and symptoms of sepsis are more varied than the initial criteria established in 1991 [2].

Although the clinical manifestations of systemic inflammation are variable, it is possible that the biochemical features are more consistent. For instance, elevated levels of circulating interleukin-6 (IL-6), adrenomedullin, soluble CD14, soluble endothelial-leukocyte adhesion molecule-1, macrophage inflammatory protein-1α (MIP-1α), extracellular phospholipase A_2, and C-reactive protein are some of the biochemical markers that have been described to be elevated in SIRS [3]. However, large-scale epidemiological studies are needed to establish these markers as confirmative indices of SIRS. Until such a time, these markers remain relevant as laboratory and research biochemical and immunological criteria to identify the inflammatory response.

Acute septic shock syndrome occurs suddenly, and patients die within 24–48 h. In severe sepsis, patients display signs of systemic inflammation and organ dysfunction, with post mortems of severe sepsis victims showing only minimal signs of inflammation or necrosis, indicating that this is a disease of low-grade inflammation [4,5]. Some patients with severe sepsis will eventually develop septic shock; thus, both severe sepsis and acute septic shock are two stages of the same disease and may occur in the same patient, but at different time periods. Therefore, it is likely that the

Address for correspondence: Undurti N Das, UND Life Sciences, 13800 Fairhill Road, #321 Shaker Heights, OH 44120, USA.
Email: undurti@hotmail.com

specific causative mediators of severe sepsis and septic shock are distinct.

In acute septic shock, a sudden overproduction of TNF-α triggers hypotension, circulatory collapse, renal and hepatic failure, and widespread inflammation and injury [6,7]. These events occur so suddenly and abruptly that there is very little time to generate sufficient amounts of neutralizing TNF-α antibodies. In contrast, in severe sepsis, plasma concentrations of TNF-α are low and anti-TNF-α antibodies may actually be harmful [8,9]. High-mobility group box-1 (HMGB1) appears to play a crucial role in severe sepsis. It is released by activated macrophages, monocytes, and a variety of other cells and is capable of inducing the release of pro-inflammatory cytokines from immune cells resulting in epithelial cell barrier dysfunction, lung injury, fever, and lethality, but not shock [10,11]. Experimental animals that die due to the effects of HMGB1 showed minimal pathological changes at necropsy, similar to those associated with severe sepsis in humans. In addition, antibodies to HMGB1 protected experimental animals against severe sepsis [10]. These results suggest that TNF and HMGB1 serve as prototype inflammatory mediators in acute septic shock and severe sepsis, with "early" and "late" patterns of expression that correlate with the acute and delayed phases of sepsis, respectively. In animal models of severe sepsis and acute septic shock, HMGB1 and TNF-α appear to play a critical role; however, there are no definitive clinical data in humans with severe sepsis or acute septic shock at present showing the beneficial effects of methods that interfere with TNF-α and HMGB1.

It should be mentioned here that both TNF-α and HMGB1 are therapeutic targets for the treatment of a variety of infections and inflammatory disorders, for example, rheumatoid arthritis and ulcerative colitis, which underscores their role as important mediators of inflammation in disease. In this context, the altered glucose uptake, utilization, and metabolism in various tissues during sepsis may be a promising pathway to consider. Recent evidence suggests that pyruvate, an intermediate product of glucose metabolism, could be of benefit both in severe sepsis and acute septic shock as it is able to block production of TNF-α and HMGB1, and that of other derived mediators which appear to play a significant role in inflammation.

Altered glucose metabolism and utilization in sepsis

Insulin resistance is common in sepsis, liver cell failure, multi-organ dysfunction syndrome, and other critical conditions. Macrophage migration inhibitory factor (MIF) is secreted, along with insulin, from pancreatic β-cells and acts as an autocrine factor to stimulate insulin release [13]. The expression of MIF in adipocytes can be modulated by insulin and glucose [12]. During the systemic inflammatory process, MIF is secreted from the pituitary gland along with glucocorticoids. The increase in plasma glucose level that occurs as a result of this glucocorticoid production is likely to be controlled by the positive effect of MIF on insulin secretion. TNF-α, whose plasma levels are increased in sepsis, induces insulin resistance by decreasing insulin-mediated whole body glucose uptake and by increasing *IL-18* gene expression in muscle tissue [14,15]. It is possible that the function of TNF-α may be to prevent hypoglycemia in the event of inappropriate insulin secretion. Thus, glucose homeostasis during stress and inflammation is maintained by glucocorticoids, TNF-α, MIF, and insulin. Furthermore, MIF expression can be induced by TNF-α, suggesting that there is a close interaction between MIF and TNF-α [16]. It is interesting to note that glucose administration is also able to inhibit TNF-α production [17]. An *in vivo* study reported that insulin was able to revert to normal the TNF-induced reduction in food intake and decrease in body weight, interstitial pneumonitis, periportal inflammation in the liver, and increases in the weights of the heart, lungs, kidney and spleen [18]. The feedback control between MIF, TNF-α, glucose, and insulin [12,16–19] suggests that administration of a glucose–insulin–potassium regimen may benefit patients with sepsis and other critically ill subjects by inhibiting MIF, TNF-α, and inducible nitric oxide (iNO) production and effects, and by augmenting endothelial NO (eNO) synthesis [20]. In animal studies of sepsis, it was noted that inhibition of MIF improved survival only when the treatment was started very early during the pathological process.

It has been shown that the glucose utilization rate is increased significantly upon exogenous insulin infusion in control subjects but not in patients with sepsis [21]. A total lack of response to the elevated levels of insulin and a decrease in whole-body glucose uptake were noted in patients with sepsis. In contrast, in the early phase of sepsis, increased tissue glucose uptake was observed. Thus, in the early phase of sepsis, hyperglycemia occurs whereas hypoglycemia or euglycemia is observed in the late stages. During the euglycemic and hypoglycemic stages of sepsis, glucose uptake remained elevated and was independent of changes in plasma glucose and insulin [22]. During sepsis, the increased level of glucose that is taken up by tissues is preferentially metabolized to lactate. In the critically ill, basal serum lactate, glucose, and insulin levels were reported to be elevated compared with control subjects and correlated with indices of severity of illness such as Acute Physiology and Chronic Health Evaluation II (APACHE II) score. Furthermore, serum glucose, free fatty acids (FFA), glycerol, and triacylglycerol, very low-density lipoprotein, and low-density lipoprotein (LDL) were elevated, whereas high-density lipoprotein cholesterol was decreased in sepsis patients

compared with those without sepsis. Elevated serum lactate and free glycerol (an indicator of lipolysis) correlated with poor survival in patients with sepsis [23]. These results suggest that both glucose and lipid metabolism are altered in sepsis secondary to impaired insulin action, in both the skeletal muscle and liver. This impaired insulin action has been attributed to decreases in insulin-stimulated phosphorylation of insulin receptor, insulin-receptor substrate-1, and mitogen activated protein kinase by endotoxin, increased clearance of insulin, and production of corticosteroids [19,20,24–27].

In the early phase of sepsis, hyperglycemia occurs due to enhanced corticosterone and normal insulin levels, whereas hypoglycemia is observed in the late stages as a result of increases in the concentrations of insulin, glucagon, and corticosterone levels by 40-fold, 6.5-fold, and six-fold, respectively [19,27]. These data suggest that blood glucose levels depend to a large extent on the balance between corticosterone and insulin levels [28], and the occurrence of initial hyperglycemia and late stage hypoglycemia in sepsis reflects altered equilibrium between MIF, TNF-α, HMGB1, corticosteroids, insulin, glucagon, and their actions. This implies that continuous infusion of glucose and insulin (with potassium to prevent hypokalemia) might enhance tissue glucose uptake, suppress lactate, FFA, glycerol production, and lipolysis, overcome corticosterone-dependent insulin resistance, enhance eNO production, and improve tissue perfusion and recovery in sepsis and the critically ill [19,29,30]. This suggestion is supported by the observation that intensive insulin treatment (~50 U/day of insulin) reduced morbidity and mortality rates among critically ill patients in the surgical intensive care unit [31,32]. This beneficial action has been attributed to the anti-inflammatory nature of insulin [19,33,34].

In this context, it is interesting to note that pyruvate has been found to be of benefit in the prevention and treatment of critical illness due to burns, ischemia/reperfusion injury, and hemorrhagic shock. Pyruvate scavenges reactive oxygen species (ROS), inhibits TNF-α and HMGB1 production, and suppresses messenger RNA (mRNA) expression of nuclear factor-κB (NF-κB), COX-2, iNO synthase (iNOS), and IL-6 in the liver, ileal mucosa, and colonic mucosa – target organs that are generally involved in sepsis, severe sepsis, and septic shock – in animal models of endotoxemia. Furthermore, pyruvate is a secretagogue of insulin. Hence, in the presence of adequate amounts of pyruvate, insulin secretion will be increased such that glucose utilization by the tissues is enhanced. As both insulin and pyruvate have anti-inflammatory actions, this will also facilitate suppression of inappropriate inflammation seen in sepsis. These results suggest that a derivative of pyruvate, ethyl pyruvate (EP), may be useful in the treatment of the critically ill and those with sepsis/septic shock; these actions are described in detail below.

Pyruvate as a free radical scavenger

Pyruvic acid ($CH_3COCOOH$) is a three-carbon α-keto monocarboxylic acid. It is present in the cells and extracellular fluids as its conjugate anion, pyruvate. As the final product of glycolysis and the starting substrate for tricarboxylic acid cycle, pyruvic acid plays a crucial role in intermediary metabolism. Pyruvate is unstable in solution, and spontaneously undergoes condensation and cyclization [35]. EP, a derivative of pyruvic acid, is not only stable and non-toxic in a calcium- and potassium-containing balanced salt solution (Ringer EP solution) [35], but is a more effective anti-inflammatory molecule than pyruvate [36]. It is extensively used as a food additive, and is approved by the US Food and Drug Administration (FDA) for this purpose. EP is used in a calcium-containing solution as it is a hydrophilic compound and the calcium prevents its emulsion and increases its solubility. As EP is chemically related to lactate and lactate is used as an inert compound in Ringer's solution, it has been suggested that lactate should be substituted with EP to provide a therapeutic anti-inflammatory component to the Ringer's solution. In general, sodium pyruvate is unstable and undergoes degradation to give rise to harmful products such as parapyruvate. Furthermore, sodium pyruvate has to be infused in large amounts that can cause hypernatremia, which is harmful. Hence, EP, derived from ethanol and pyruvic acid, in a Ringer-type Ca^{2+}- and K^{+}-containing balanced salt solution, is both stable and more effective than sodium pyruvate [37].

Ringer EP solution prevented both structural and functional damage induced by mesenteric ischemia/reperfusion injury to the intestinal mucosa, ameliorated hepatic and intestinal mucosal lipid peroxidation, and prolonged survival by decreasing intestinal mucosal injury induced by hemorrhagic shock in rats [38]. These results suggest that EP is an effective free radical scavenger. Further studies revealed that pyruvate could also scavenge hydroxyl (OH) radicals as evident from the observation that pyruvate prevented mucosal injury induced by ischemia/reperfusion to rat small intestine, myocardial damage inflicted by ischemia/reperfusion, and renal injury induced by H_2O_2 in experimental animals [39–42]. Pyruvate prevented galactose or diabetes-induced cataract formation both *in vitro* and *in vivo*, and stroke and hemorrhagic shock, which are effects that could be attributed to anti-inflammatory actions [43–47]. These studies confirm that pyruvate suppresses free radical-induced tissue damage, and, by virtue of its free radical scavenger property, it could be of significant benefit in sepsis, in which free radicals are known to play a dominant role.

EP in hemorrhagic shock

In a rat model of severe hemorrhagic shock, both pre-treatment and post-treatment (in the form of resuscitation

fluid) with a solution of Ringer's EP solution ameliorated gut mucosal damage in animals subjected to mesenteric ischemia/reperfusion. EP also decreased lipid peroxidation and improved survival by obliterating ileal mucosal permeability (a feature that is also seen in sepsis and septic shock) in these animals, suggesting that EP scavenges reactive oxygen species (ROS) [46]. Woo et al. reported that EP decreased infarct size and lipid peroxidation in a rat model of transient coronary artery occlusion followed by reperfusion [48]. In that study, EP administration significantly increased myocardial adenosine triphosphate levels, reduced oxidative injury, diminished infarct size, and improved myocardial function compared with control animals. In contrast, Mulier et al. found no significant beneficial effects of EP in an animal model of hemorrhagic shock [49]. These contrasting results could be due to the differences in the types of animal models used and dose of EP employed. Despite these results, several other studies have demonstrated that EP has potent anti-inflammatory and free radical quenching actions, which suggest that EP could be of benefit in sepsis [50].

EP ameliorates sepsis and other inflammatory conditions

An early TNF-α and IL-1 and late HMGB1 pro-inflammatory cytokine response to infection, injury, or surgery mediates sepsis. EP has been shown to attenuate lethal systemic inflammation caused by either endotoxemia or sepsis even when the treatment was started after the early TNF-α response [50]. Administration of EP 24 h after cecal ligation and puncture (an experimental model of polymicrobial sepsis), significantly increased survival and reduced circulating levels of TNF-α and HMGB1. *In vitro* studies showed that EP inhibited the release of TNF-α and HMGB1 from endotoxin-stimulated murine macrophages and attenuated activation of NF-κB signaling pathways. In an LPS-induced endotoxic shock model, EP improved survival and lowered circulating concentrations of nitrite/nitrate (metabolites of NO) and IL-6, which are pro-inflammatory molecules, and enhanced plasma levels of IL-10, an anti-inflammatory cytokine, suggesting that EP has significant anti-inflammatory actions [51].

In an *in vitro* study, exposure of Caco-2 human enterocytic cells to interferon-γ, TNF-α, and IL-1β increased permeability to fluorescein isothiocyanate-labeled dextran, NF-κB activation, iNOS mRNA expression, NO production, and modulated expression and localization of the tight junctional proteins, zonula occludens-1, and occludin; these effects were inhibited by EP. EP was also found to ameliorate ileal mucosal hyperpermeability and bacterial translocation to mesenteric lymph nodes in LPS-challenged mice, suggesting that it is capable of preserving intestinal epithelial barrier function disturbed by cytokines and LPS [52].

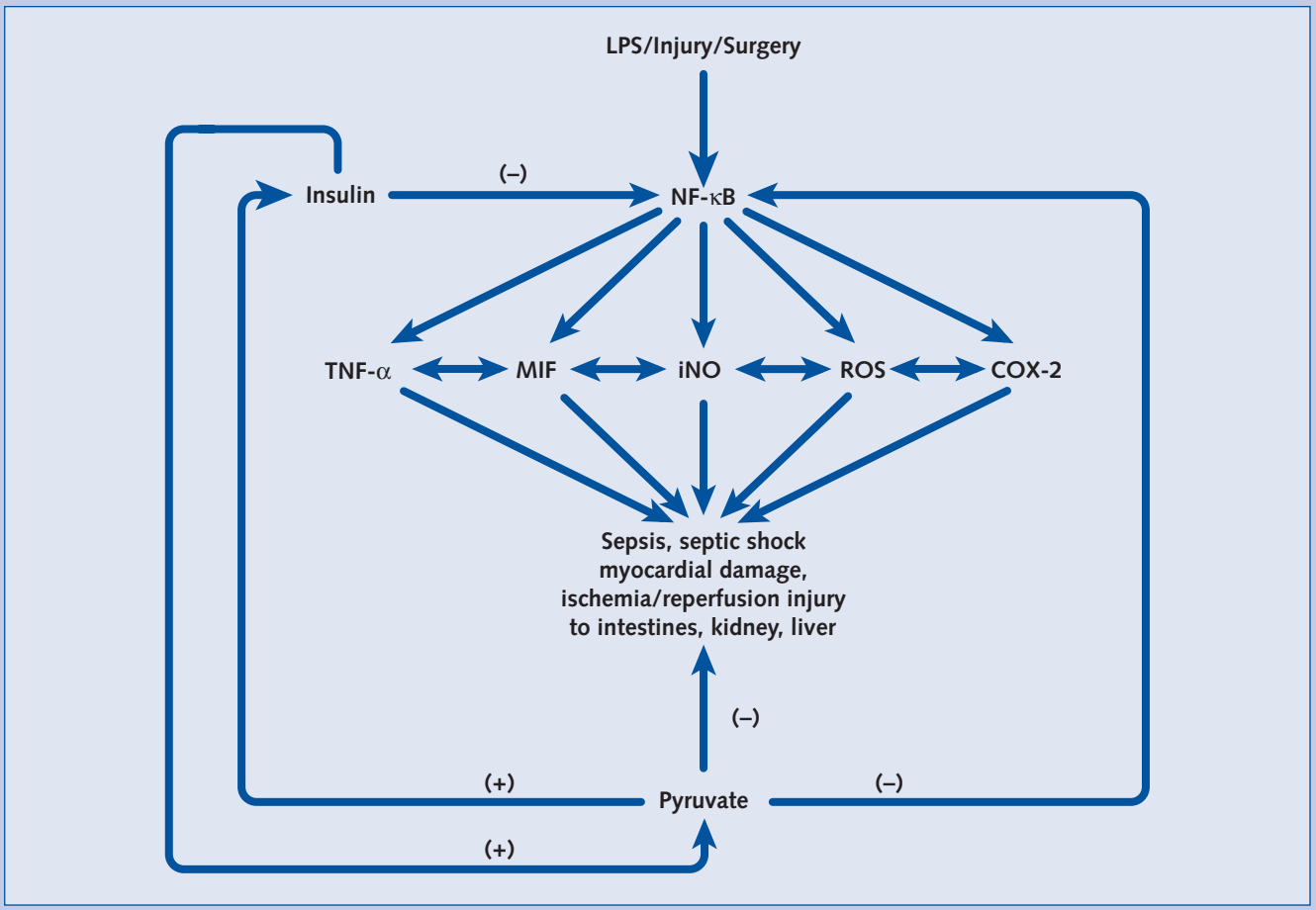
Acute alcohol intoxication and extrahepatic cholestasis mediate increases in lipid peroxidation, NF-κB activation, and TNF-α mRNA expression. The resultant hepatic inflammatory response and hepatocellular injury are prevented by EP [53–56]. Furthermore, EP decreased gut manipulation-induced increases in IL-6 and iNOS mRNA levels and restored intestinal smooth muscle contractility [57].

Insulin resistance and pyruvate: application to sepsis

Insulin can influence the production of pyruvate by its modulatory action on glucose metabolism, while pyruvate is an insulin secretagogue [58]. Hence, it is reasonable to suggest that in instances of insulin resistance and defective insulin action, the production and actions of pyruvate would also be defective or suboptimal.

For instance, lipid oxidation is increased in obesity and type 2 diabetes mellitus. Increased lipid oxidation suppresses glucose oxidation due to decreased glucose uptake in skeletal muscle, and this leads to the development of insulin resistance. The products of FFA, which are formed as a result of lipid oxidation, catalyze a key step of glucose oxidation, namely, the conversion of pyruvate, formed during glycolysis, to acetyl-coenzyme A (acetyl-CoA). The products of FFA oxidation (acetyl-CoA and nicotinamide adenine dinucleotide) regulate the pyruvate dehydrogenase (PDH) enzyme complex that converts pyruvate to acetyl-CoA. The PDH complex is inhibited by phosphorylation of one of its components by pyruvate dehydrogenase kinase (PDK). Increased PDK activity suppresses glucose oxidation and induces insulin resistance in subjects with obesity and type 2 diabetes. Both starvation and experimental diabetes induce an increase in PDK activity in skeletal muscle, which leads to a reduction of glucose oxidation. Insulin suppresses PDK-4 (an isoform of PDK that is expressed in skeletal muscle) and thus, enhances glucose oxidation by enhancing the conversion of pyruvate to acetyl-CoA [59]. Zucker fatty (ZF) rats, which are obese, hyperlipidemic, and normoglycemic, showed a 3.8-fold increased β-cell mass along with a 3–10-fold increase in insulin secretion in response to various stimuli, due to a 250% increase in pyruvate in mitochondria that accounted for increased mitochondrial glucose metabolism [60]. This suggests that availability of enhanced amounts of pyruvate stimulates the production of insulin and thus, prevents the development of hyperglycemia in ZF rats. In this context, it is interesting to note that obese subjects were found to have decreased PDH activity, leading to decreased glucose oxidation, which is attenuated when plasma insulin is enhanced [61,62]. These data suggest that in subjects with obesity, type 2 diabetes mellitus, or metabolic syndrome X, the production of pyruvate is decreased due to decreased PDH activity, which is the rate-limiting enzyme that irreversibly

Figure 1. Scheme showing the relationship between various mediators of sepsis/septic shock, insulin, and pyruvate. The scheme summarizes the actions of pyruvate and the mediators involved in sepsis.



(–): indicates inhibition of synthesis/action/negative feedback control; (+): indicates augmentation of action/synthesis/positive feedback control; COX-2: cyclooxygenase-2; iNO: inducible nitric oxide; LPS: lipopolysaccharide; MIF: macrophage inhibitory factor; NF-κB: nuclear factor-κB; ROS: reactive oxygen species; TNF-α: tumor necrosis factor-α.

channels intermediate metabolites from anaerobic glucose breakdown to either oxidative metabolic pathways or fatty acid and cholesterol synthesis. This leads to insulin resistance, which, in turn, causes decreased PDH activity. The decreased intracellular pyruvate levels could account for the high incidence of sepsis in these conditions. As pyruvate has anti-inflammatory actions, this may also explain why pro-inflammatory markers are enhanced in these conditions [63]. In view of the beneficial actions of EP in animal models of hemorrhagic shock, sepsis, and myocardial ischemia/reperfusion injury, it remains to be seen whether EP or its structural analogues alone or with insulin, which also has anti-inflammatory effects [19,20], would form a new approach in the treatment of these conditions.

Conclusion

From the preceding discussion, it can be assumed that EP protects the myocardium, intestines, hepatic, and renal tissues from ROS-, cytokine-, and ischemia/reperfusion-induced injury (Fig. 1). Indeed, EP has the ability to inhibit binding of NF-κB to DNA, decrease iNO production, quench ROS, prevent the production of IL-1, IL-6, COX-2, TNF-α, MIF, and HMGB1, and simultaneously enhance expression of the anti-inflammatory cytokine IL-10. However, it is not yet known whether there is altered production and utilization of pyruvate in sepsis. Insulin, by virtue of its action on glucose metabolism, influences the production of pyruvate, while pyruvate is an insulin secretagogue. This indicates that in instances of insulin resistance and defective insulin action, for example, in sepsis and the other conditions described in this review, the production and actions of pyruvate may be defective or suboptimal. In view of this, it will be interesting to study whether the altered insulin dynamics seen in sepsis would lead to changes in pyruvate production and action.

EP is already in Phase II clinical trials under the trade name CTI-01 and in one sepsis-related trial to determine its efficacy and identify possible side effects.

Disclosure

Dr Das has no relevant financial interests to disclose.

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Glycemic Control in Sepsis

Armelle Mathonnet and Alain Cariou

Medical Intensive Care Unit, Cochin Hospital AP-HP, Paris 5 René Descartes University, Paris, France

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During 2004, the experts involved in the Surviving Sepsis Campaign (SSC) established guidelines for the management of severe sepsis and septic shock. Besides well-established procedures such as anti-infectious and hemodynamic management, several adjunctive therapies (e.g. steroids, recombinant human activated protein C, glucose control) were also considered in order to improve metabolic control and diminish the risk of organ dysfunction [1]. In this setting, the importance of controlling endocrine disorders is now well recognized. Indeed, the endocrine system is frequently affected during sepsis, and may also provoke other systemic disorders.

Septic patients may develop hyperglycemia due to a combination of several factors. Firstly, insulin clearance is increased leading to a reduction in insulin-mediated glucose uptake [2]. Moreover, stress induces an elevation in plasma levels of counter-regulatory hormones, such as catecholamines, glucagon, cortisol, and growth hormone. Consequently, hepatic glycogenolysis is promoted and hepatic gluconeogenesis is increased [2]. Adverse effects of several therapies (such as glucocorticoids or sympathicomimetic drugs) may also contribute to the development of hyperglycemia in septic shock [2,3]. Furthermore, insulin resistance, which is proportional to the severity of stress response, worsens hyperglycemia. This insulin resistance could be due to a defective GLUT4 transporter and to the deleterious effects of the pro-inflammatory cytokines interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α). As an illustration, TNF-α decreases the expression and phosphorylation of insulin cell surface receptors, leading to liver adipocyte and muscle insulin resistance [2–4]. Together, these abnormalities may explain why hyperglycemia and insulin resistance are common in septic patients, even in those who did not previously have diabetes [5–8].

Historically, hyperglycemia was considered to be an adaptive response, providing glucose for the brain, red blood cells, and wound healing, and was treated only when blood glucose levels were >215 mg/dL (>12 mmol/L) [2]. However, recent results of experimental and clinical research underscored the beneficial effects of treating hyperglycemia at an earlier time point. It was hypothesized that, in critically ill patients, hyperglycemia and insulin resistance may confer a predisposition to several complications such as severe infection, polyneuropathy, multi-organ failure, and death [2,3]. This concept was mainly an extrapolation of well-known consequences of chronic hyperglycemia in diabetic patients [7,8]. In critically ill patients, increased oxidative stress and bioenergetics failure contribute to multiple organ failure. The dysfunctional mitochondrial respiration seen in sepsis can affect all organs and may lead to overproduction of superoxide. Van den Berghe et al. showed that restoration or maintenance of mitochondrial function and cellular energetics, observed when normoglycemia is maintained with intensive insulin therapy, may improve outcomes for critically ill patients [9].

These observations led to several questions regarding the optimal blood glucose level to be maintained in critically ill patients. In 2001, Van den Berghe et al. published the results of a randomized, controlled study that investigated the impact of intensive insulin therapy in mechanically ventilated patients treated in their surgical intensive care unit (ICU) [10]. Patients were randomized to receive either intensive or conventional insulin therapy. In the intensive therapy arm, the blood glucose concentration was firmly maintained in the range of 80–110 mg/dL. This study showed that strict control of blood glucose levels with insulin significantly reduced morbidity and mortality rates. Indeed, intensive insulin therapy reduced the ICU mortality rate by 43% and in-hospital mortality rate by 34%. A shorter duration of mechanical ventilation and ICU stay was also observed with intensive glycemic control. The incidence of infections was reduced by 46% and significant differences were observed in the occurrence of acute renal failure requiring renal replacement therapy and the need for red blood cell transfusions. The authors of this study provided several explanations for these benefits including: prevention of immune dysfunction, reduction of systemic inflammation,

Address for correspondence: Armelle Mathonnet, Medical Intensive Care Unit, Cochin Hospital, 27, rue du Faubourg Saint Jacques, 75679 Paris Cedex 14, France. Email: armelle.mathonnet@cch.aphp.fr

protection of the endothelium, and protection of mitochondrial ultrastructure and function [9].

Following this publication, questions arose as to whether the benefits were the direct consequence of the infused insulin *per se* or were obtained by the prevention of hyperglycemia, as both occurred concomitantly. Insulin has been shown to inhibit TNF-α [11], and it is also likely that the infusion of glucose and insulin inhibits macrophage inhibitory factor [12]. Thus, the improved outcomes observed in the group receiving intensive insulin therapy may have resulted primarily from the action of insulin on these cytokines rather than from the relatively mild hyperglycemia that was observed in the conventional treatment group. In summary, insulin might have had a role independent of its effect on glycemia. All of the effects of insulin could also contribute to the well-documented benefits of treating hyperglycemia to reduce infection. Besides the anti-inflammatory effects of insulin [13], favorable effects on coagulation and fibrinolysis [14,15] and on macrophage function [16], partially mediated by the prevention of hyperglycemia, may also have occurred. Given the practical complexity involved in maintaining normoglycemia in critically ill patients and the potential dangers associated with these attempts, it appeared questionable to wholly attribute these results to the normalization of blood glucose levels.

In an effort to resolve this issue, Van den Berghe et al. analyzed data from their previous study [10]. Multivariate logistic regression analysis revealed that the daily dose of insulin and mean blood glucose level were independent positive predictors of mortality in the study population. Metabolic control, as reflected by normoglycemia rather than the infused insulin dose *per se*, was related to the beneficial effects of intensive insulin therapy. Thus, a higher dose of insulin was associated with a worse outcome, and a lower blood glucose level was associated with a better outcome, suggesting that the latter had a crucial role [17].

Some experts also argued that the benefits observed with intensive insulin therapy were due to the prevention of hyperglycemic complications related to parenteral nutrition, and questioned whether the intensive insulin regimen was beneficial in patients who did not receive parenteral nutrition. Van den Berghe et al. showed that a gradual transition from intravenous nutritional support to enteral nutrition resulted in the administration of similar numbers of calories and similar amounts of glucose, protein, and lipids in both treatment groups at all times [10]. The mortality rate was reduced to 68% in the group that received intensive insulin therapy associated with enteral nutrition, and to 60% in the combined parenteral–enteral feeding group. A subsequent observational study also suggested that insulin therapy improved outcome in a single-center medical and surgical ICU [18].

On the basis of these results, several groups recommended that glycemic control with intensive insulin therapy should become standard care for the critically ill patient. The Joint Commission on Accreditation of Healthcare Organization (www.jcaho.org) recently proposed tight glucose control for critically ill patients as a core quality of care measure for all US hospitals that participate in the Medicare program. Finally, during the establishment of the SSC guidelines, the committee experts recommended glycemic control, although only a grade D level of evidence was attributed to this recommendation [1]. The SSC experts considered that the extrapolation of benefits observed in the surgical population was not firmly established in medical patients, particularly in the septic population where the risk of hypoglycemia in sepsis was considered high. Indeed, severe hepatic dysfunction and renal failure are also more frequent and may lead to more frequent treatment adverse effects. Additional arguments came from the results of a recently published study. Van den Berghe et al. conducted a prospective, randomized, controlled study of adult patients in a medical ICU [17]. On admission, patients were randomly assigned to strict normalization of blood glucose levels (80–110 mg/dL [4.4–6.1 mmol/L]) with the use of insulin infusion or to conventional therapy (insulin administered when blood glucose levels exceeded 215 mg/dL [12 mmol/L], with the infusion tapered when levels fell below 180 mg/dL [10 mmol/L]). In the intention-to-treat analysis of 1200 patients, intensive insulin therapy reduced blood glucose levels but did not significantly reduce in-hospital mortality rates. However, morbidity was significantly reduced in the intensive glycemic control arm by prevention of newly acquired kidney injury, accelerated weaning from mechanical ventilation, and accelerated discharge from the ICU and hospital. Interestingly, among the 433 patients who stayed in the ICU for <3 days, the mortality rate was greater in those receiving intensive insulin therapy. In contrast, of the 767 patients who stayed in the ICU for ≥3 days, 386 received intensive insulin therapy; in this subgroup, the in-hospital mortality rate was reduced from 52.5% to 43.0% (p=0.009), and morbidity was also decreased. The main conclusion of this study was that intensive insulin therapy during intensive care did not significantly reduce the risk of death among all patients in the medical ICU included in the intention-to-treat population. However, among those who stayed in the ICU for ≥3 days, intensive insulin therapy reduced morbidity and mortality rates. Regarding the issue of intensive insulin therapy specifically in septic patients, the question remains unresolved by this study, as it included only a small number of sepsis cases [10,18,19].

A recent German multicenter study testing intensive insulin therapy in patients with severe sepsis was suspended by the safety monitoring board due to a significant excess risk of severe hypoglycemia, without any evidence of improved survival [20]. As this study did not provide information regarding efficacy (because it was underpowered to detect an effect on mortality), the role of intensive insulin treatment in the setting of sepsis remains unclear. In the meantime, we await the outcomes of two ongoing, large-scale, multicentric, randomized trials examining the issue of glycemic control in the ICU. The first is the Glucontrol (A Multi-Center Study Comparing the Effects of Two Glucose Control Regimens by Insulin in Intensive Care Unit Patients) study, which randomized 3500 medical and surgical ICU patients in several European centers to one of two different insulin regimens, designed to achieve either tight or modest blood glucose control [21]. The second trial is the NICE-SUGAR (Normoglycaemia in Intensive Care and Evaluation and Survival Using Glucose Algorithm Regulation) study, a multicenter, open-label, randomized, controlled trial of two target ranges for glycemic control in ICU patients [22]. Neither of these studies mandates glucose infusions or parenteral nutrition, and both are intended to enroll a broad case-mix of patients from a large number of ICUs.

It now seems important to develop a system for continuous glycemic monitoring before considering intensive insulin therapy for critically ill patients. These continuous glucose-monitoring systems are eagerly awaited as they could reduce the occurrence of adverse events with intensive insulin therapy [23]. Finally, any strategy of strict glycemic control should be carefully coordinated with the level of nutritional support and metabolic status, which change frequently in septic patients.

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Letter to Surviving Sepsis Campaign Network Heads and Sponsoring Organization Leadership

Mitchell M Levy on Behalf of the Surviving Sepsis Campaign Steering Committee Members

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As you may know, the October 19, 2006 issue of the *New England Journal of Medicine* contains an opinion piece entitled "Surviving Sepsis—Practice Guidelines, Marketing Campaigns, and Eli Lilly" by Peter Eichacker, Charles Natanson, and Robert Danner [1]. The commentary takes the position that the Surviving Sepsis Campaign (SSC) and the associated guidelines have been influenced by Eli Lilly. Unfortunately, in the authors' zeal to address an alleged improper relationship with Eli Lilly, the power, evolution, successes and other robust interventions included in the Campaign are not acknowledged. The SSC is the driving force behind a phenomenal change in the quality of care provided to severely septic patients. Without the campaign, there would be no effort to reduce relative risk of mortality from severe sepsis by 25% globally within 5 years.

The members of the SSC executive committee wish to assure you that we take very seriously the implication that the guidelines reflect anything less than the best practices and evidence available at the time they were developed. From the Campaign's inception, the role of industry in the Campaign has been clearly and frequently stated to all publics. Transparency about the role of industry in providing financial support for the Campaign as well as the relationships with industry of all the people involved in the Campaign's leadership and of those who participated in the development of the guidelines has always been clearly stated. In fact, the guidelines themselves describe the relationship in great detail:

"This process [guidelines development] represented phase II of the Surviving Sepsis Campaign, an international effort to increase awareness and improve outcome in severe sepsis. Meeting expenses as well as staff support for guidelines creation were provided by unrestricted industry educational grants as listed. There were no industry members of the committee. There was no industry input into guidelines development and no industry presence at any of the meetings. Industry awareness or comment on the recommendations was not allowed. The sponsors of the educational grants did not see the recommendations until the manuscript was peer reviewed and accepted for publication in final form" [2].

The Campaign leadership is acutely aware of the inherent risk of misinterpretation when industry is involved in science. Nevertheless, the benefits to the public from the power of partnerships far outweigh the criticisms that accrue from outside the Campaign. The organizations involved had to weigh the idea of a Campaign supported by industry versus not having the activity at all. Funding from other sources, such as the National Institues of Health (NIH), was not forthcoming and not likely to be in the near future. So if the effort to reduce mortality from severe sepsis by raising awareness and standardizing treatment were to occur, it had to happen via commercial support.

Always concerned about transparency in the funding process as a safeguard against the type of influence intimated by the authors, the SSC has produced an "industry fact sheet" outlining the Campaign's position on the use of industry funds – both by the Campaign and the sites seeking to implement the Campaign. This fact sheet is distributed at every event held by the SSC.

To address some of the specific items mentioned in the authors' opinion piece, we wish you to be aware that there are factual errors in the description of the founding of the Campaign and the Campaign's relationship to the public relations firm Belsito and Company. Although much of this will be addressed in a forthcoming reply in print, it is important to note that the Campaign was established prior to the timeline cited by the authors. Moreover, the Campaign itself hired Belsito and Company to assist with promoting the campaign, not Eli Lilly. Nobody should be surprised that a nascent campaign required the efforts of a public relations firm to spread its aims, goals and intentions.

Many of the concerns cited by the authors in their commentary about the involvement of industry in guidelines development dissolve with the upcoming publication of the 2006 guidelines revision. While the Campaign vigorously defends the position that no impropriety existed in the use of industry funds to hold a meeting to establish the 2004 guidelines, all involved were acutely aware that if the meeting could be held without industry funding concerns about undue industry influence would be allayed. To that end, the 2006 revision of the guidelines was supported by the Society of Critical Care Medicine with no industry funding. Delegates traveled to this meeting either at their own expense or that of the society that sponsored their participation. All items included in the revised guidelines were subject to a private ballot for acceptance or rejection. With respect to recombinant human activated protein C (rhAPC), 84% of the 52-member committee approved the new recommendation, which continues to identify its place in the treatment of severe sepsis. The results of the non-industry funded guidelines revision in 2006 confirm that the 2004 guidelines committee had acted appropriately to include the use of rhAPC in the treatment of patients with severe sepsis.

Given the controversies surrounding rhAPC, the Campaign made a strategic decision that the performance improvement program offered to hospitals – the bundles and measures – would not mandate the use of rhAPC. In fact, the bundles that the authors cite only require that hospitals create and adhere to a standardized policy on the administration of rhAPC. The assumption is that physician experts can assess the guideline's recommendation and rationale as well as the literature on which it is based and create a rational use policy. Indeed, the standard policy could be non-administration of rhAPC and the hospital would receive full credit for compliance with this measure. In addition, the revision of the guidelines incorporates and cites all of the available science on rhAPC noted by the authors in their opinion piece.

The SSC is a novel and creative attempt to change behavior at the bedside in an effort to improve patient care. Hospitals that are participating and the organizations that provided time and volunteer resources to develop the guidelines should be proud of the contribution they are making. Never before have guidelines for practice been introduced in this manner with the development of care bundles and an organized method of collecting and aggregating data to document and provide feedback to enable quality improvement. The data that have been collected so far are proof that this effort is succeeding. As the Campaign leadership, we are firmly committed to continuing our efforts and providing positive messages about activities that ultimately contribute to the greater good.

We welcome your comments, questions, and concerns and are committed to an open, productive discussion of this issue.

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CLINICAL REVIEWS

Commentary and Analysis on Recent Key Papers

Clinical reviews were prepared by Daniel De Backer, Nicholas Ward, and Eric Wiel

CLINICAL OBSERVATIONS AND RESEARCH

Adrenal axis function does not appear to be associated with hemodynamic improvement in septic shock patients systematically receiving glucocorticoid therapy

Morel J, Venet C, Donati Y et al.
Intensive Care Med 2006;**32**:1184–90.

This study aimed to determine the predictive value of adrenocorticotrophic hormone stimulation tests in identifying those patients with septic shock that would benefit from treatment with exogenous steroids. A retrospective assessment of 52 patients who received steroids found that an abnormal test did not predict hemodynamic improvement with steroids.

In recent years, much research has focused on the significance of the hypothalamic–adrenal axis in patients with septic shock. One large study showed that administration of exogenous glucocorticoids in hypotenisve patients improved hemodynamics and outcomes [1]. This and other studies have suggested that patients with an abnormal adrenocorticotrophic hormone (ACTH) stimulation test may benefit from exogenous steroids to a greater degree than those with a normal test.

The investigators of the present study performed a retrospective assessment of 52 consecutive patients with septic shock who received exogenous steroids in order to determine if an abnormal ACTH stimulation test predicted hemodynamic improvement with steroids, which they defined as a reduction in the dose of vasopressors of ≥50%. They found that 55.8% of the patients improved in the first 3 days, but that having an abnormal stimulation test did not predict which patients responded to steroids. Responders and non-responders did not differ significantly in terms of infection site, organism, or management.

This study is in agreement with several other studies that have shown that abnormal ACTH stimulation tests do not predict greater success with the use of steroids in septic shock. One confounding factor in these studies is the inconsistency with which an abnormal test is defined. Another factor is that it is likely that many patients will improve, despite receiving steroids. Clearly, additional, large studies are needed before the value of testing cortisol stimulation is understood.

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Address for Reprints: F Zeni, Polyvalent Intensive Care Unit, Hospital Bellevue, 42055 Saint Etienne Cedex 2, France.
Email: zeni@univ-st-etienne.fr

Persisting low monocyte human leukocyte antigen-DR expression predicts mortality in septic shock

Monneret G, Lepape A, Voirin N et al.
Intensive Care Med 2006;**32**:1175–83.

Sepsis is characterized by both a pro- and anti-inflammatory response. Previous studies have shown the magnitude of the anti-inflammatory response to be predictive of poor outcomes. The authors of this study sequentially followed levels of a proposed anti-inflammatory marker, monocyte human leukocyte antigen-DR, in patients with sepsis and demonstrated that a failure to increase levels of this marker after day 2 correlated with increased mortality rate.

Sepsis is usually characterized by an initial pro-inflammatory response referred to as the systemic inflammatory response syndrome (SIRS). It is now clear that shortly after this occurs, the immune system creates a compensatory anti-inflammatory response syndrome (CARS) in which several immune functions are downregulated. Accepted components of this response include the expression on interleukin-10, lymphocyte apoptosis, deactivation of monocytes, and the downregulation of the antigen-presenting DR receptor on monocytes. Previous studies have shown a correlation between the magnitude of the CARS response and poor outcomes in a variety of inflammatory conditions [1]. The authors of this study examined human leukocyte antigen-DR (HLA-DR) expression on moncytes in patients with sepsis and assessed its use as an indicator of a poor prognosis.

The investigators followed 93 patients with septic shock and measured monocyte HLA-DR expression over time. Overall, the mortality rate was 34% with the majority of the deaths occurring >48 h after the time of diagnosis. Their results showed that, in the first 2 days, there was no significant difference in HLA-DR expression between those who survived and those who did not; however, a significant difference appeared after 48 h with more non-survivors showing persistent depression of expression (48%) compared with survivors (18%). Multiple logistic regression analysis showed that an HLA-DR expression of <30% at day 3–4 was independently associated with mortality rate after controlling for confounding variables.

This study is yet another piece of clinical data suggesting that the persistence or magnitude of the CARS response is associated with a poor outcome in sepsis. This information could be useful in predicting the course of a septic patient. In the future it may also become possible to use this information to tailor pro-inflammatory therapies to these patients in order to improve outcomes.

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Address for reprints: G Monneret, Hôpital Neurologique, Immunology Laboratory, Lyon, France. Email: guillaume.monneret@chu-lyon.fr

Increased colorectal permeability in patients with severe sepsis and septic shock

Jørgensen VL, Nielsen SL, Espersen K et al.
Intensive Care Med 2006;**32**:1790–6.

The intestinal barrier has an important role in the pathophysiology of sepsis. The authors of this study developed a new method for the assessment of intestinal barrier integrity, using a marker of paracellular permeability. This method was able to demonstrate an increased permeability in the large bowel of septic patients that was correlated with the luminal concentrations of L-lactate, which is suggestive of mucosal metabolic dysfunction.

Intestinal barrier dysfunction contributes to the pathophysiology of sepsis, which itself induces regional ischemia with bacterial translocation. Several techniques have been developed to ascertain intestinal barrier integrity, but interpretation of results obtained using these methods remains difficult. Moreover, no studies have assessed mucosal permeability in the large bowel. The authors of this study developed a method to assess colorectal permeability in septic patients, and aimed to identify a link between permeability and luminal concentration of L-lactate, which is indicative of mucosal metabolic dysfunction [1].

In this clinical study performed in intensive care unit patients with sepsis, colorectal permeability was assessed by the initial appearance rate of rectally administered ^{99m}Technetium-diethylene triamine penta-acetic acid (^{99m}Tc-DTPA) in plasma. An increased rate of permeability was found in patients with severe sepsis and septic shock compared with healthy subjects. The permeability rate was correlated with both arterial and luminal concentrations of L-lactate; however, it was not positively related to the simplified acute physiology score II, mean arterial blood pressures, or bladder pressures. The authors calculated the cumulative systemic recovery of the marker at 1 h, by estimation of volume of distribution and renal clearance of ^{99m}Tc-DTPA (by comparison with the volume of distribution and renal clearance calculated using an intravenous bolus of ^{51}chromium-ethylene diamine tetra-acetic acid). An increased cumulative permeability was found in patients with severe sepsis and septic shock, compared with control subjects. This was correlated to the initial permeability rate. Some limitations of this study include the fact that patients were not matched for age or treatments (vasopressors and fluid challenge were not standardized). Nine patients with septic shock had a laparotomy, which might have induced higher intestinal permeability. Moreover, apoptosis of the intestinal epithelium may have played a role; however, this is also unclear.

In conclusion, this study demonstrated reduced barrier integrity in the large bowel of septic patients. Increased intestinal permeability was correlated with luminal L-lactate concentration, which is suggestive of mucosal metabolic dysfunction.

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Address for reprints: A Perner, University of Copenhagen, Department of Intensive Care, Rigshospitalet, 2100 Copenhagen, Denmark. Email: ap@dadlnet.dk

Erythropoiesis abnormalities contribute to early-onset anemia in patients with septic shock

Claessens YE, Fontenay M, Pene F et al.
Am J Respir Crit Care Med 2006;**174**:51–7.

The authors of this study demonstrated that apoptosis pathways, specifically Fas ligand, play a key role in the development of early-onset anemia in patients with septic shock.

Critically ill patients are frequently anemic. In addition to blood losses, altered erythropoiesis may contribute to anemia. Red blood cells are generated from pluripotent stem cells under the influence of erythropoietin, the stimulatory effect of which is counterbalanced by the inhibitory effects of ligands of tumor necrosis factor (TNF) receptor family members, in particluar, the Fas ligand and the TNF related apoptosis-inducing ligand (TRAIL). The authors investigated

erythropoiesis abnormalities in patients with early-onset anemia during septic shock.

Ten patients with septic shock related to community-acquired pneumonia who developed early-onset anemia (hemoglobin levels <10 g/dL within 48 h of admission to the intensive care unit [ICU]) were included. Patients with acute bleeding, history of bone marrow disease, chronic renal disease, HIV, hematological diseases, or immunosuppression were excluded. Healthy controls (patients undergoing bone marrow sampling for exploration of thrombophilia) were also included.

Sera of control subjects and septic patients were analyzed and cytokine levels were measured. Bone marrow was obtained within 48 h of admission to ICU (mean 30 h), the bone marrow mononuclear cells were isolated, and cellular lineages quantified. Bone marrow mononuclear cells expressing the glycophorin A (GPA) membrane receptor were isolated using immunomagnetic columns and the GPA levels quantified by flow cytometry. Bone marrow mononuclear cells were also cultured to quantify clonogenic erythroid progenitors in the presence of control or septic patients' sera. Erythropoietin was added either at a normal dose, or at a rescue dose (four times the previous dose). Erythropoietin receptor signaling was also evaluated after erythropoietin administration. Finally, apoptosis (assessed by annexin V and propidium iodide staining) and mitochondrial membrane potential were evaluated, and immunophenotyping was performed on GPA-positive cells.

In patients with septic shock, hemoglobin levels were 8.3–9.7 g/dL and red blood cell volume was normal. Reticulocyte numbers were moderately elevated (20–130 /mL) while erythropoietin levels were within a normal range (19–54 mU/mL; these would be expected to be elevated). The erythroid lineage was not decreased in the bone marrow of septic patients; however, apoptosis of bone marrow erythroid precursors was increased. Flow cytometry identified Fas ligand as being highly expressed, while TRAIL expression did not differ from controls. Addition of sera of septic patients impaired the development of erythroid progenitors.

The authors concluded that apoptotic pathways, specifically Fas ligand, play a key role in the development of anemia in patients with septic shock. Unfortunately, this study did not include a control group of subjects from the ICU or patients with septic shock who were not developing early-onset anemia. Accordingly, some of the differences between healthy controls and patients with early anemia may perhaps not be related to the development of anemia.

Address for reprints: A Cariou, Medical Intensive Care Unit, Cochin Hospital, APHP Universite Paris-Descartes, 27 rue du Faubourg Saint-Jacques, F-75679 Paris, Cedex 14, France.
Email: alain.cariou@cch.ap-hop-paris.fr

Systemic inflammatory response syndrome and nosocomial infection in trauma

Hoover L, Bochicchio GV, Napolitano LM et al.
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Criteria that indicate the occurrence of systemic inflammatory response syndrome (SIRS) are frequently observed in trauma during the first days post-injury. The authors of this study assessed the importance of the later occurrence or persistence of SIRS criteria and found these to be highly suggestive of ongoing infection.

The systemic inflammatory response syndrome (SIRS) occurs frequently in trauma patients. In these patients, SIRS is associated with the development of organ dysfunction and a higher mortality rate. Infection is a common problem in patients with trauma, occurring in close to 40% of individuals, although the differentiation of infectious versus non-infectious SIRS may be problematic. The presence of SIRS criteria on admission to an intensive care unit (ICU) is predictive of infection in blunt trauma; however, the link between SIRS and development of nosocomial infection has not previously been ascertained. The current authors investigated the use of a daily SIRS score to predict subsequent infections.

This prospective study investigated 1277 consecutive trauma patients admitted into the ICU or intermediate care area of the Maryland trauma center (University of Maryland School of Medicine, Baltimore, MD, USA) during a 26-month period. A SIRS score (one point for each of its components) was calculated daily for the first 7 days and then every other day for the subsequent 14 days. Infection was diagnosed, using Centers for Disease Control and Prevention criteria, by a multidisciplinary team that included trauma, ICU, infectious disease specialists, and, if required, a radiologist. No routine cultures were undertaken; sputum cultures and invasive sampling were reserved for patients who displayed progression of lung infiltrates.

A SIRS score of ≥2 was observed on day 1 post-injury in 92% of the patients. This incidence of SIRS decreased over time, being present in just 22% of patients on day 21. The incidence of infection was higher in patients with a SIRS score of ≥2 on day 1 post-injury (47% vs. 41%), as was the mortality rate (16% vs. 11%). The risk of nosocomial infection, assessed by an odd ratio for a SIRS score of ≥2 on a given day, was 1.28 on day 1 post-injury, and increased rapidly to 3.9 after 1 week and 24.7 after 3 weeks. Thus, SIRS criteria are frequently observed on day 1, but these should rapidly disappear. The occurrence or persistence of SIRS criteria after day 1 post-injury is highly suggestive of ongoing infection.

One of the most important limitations of the study is the manner in which infection was diagnosed. No specific sampling was undertaken, and, in many cases, SIRS criteria (i.e. fever or leukocytosis) may have been taken into account by the multidisciplinary team diagnosing infection. Ideally, these physicians should not have been aware of the SIRS criteria. Another important limitation is that there were some errors in the presentation of the data; the incidence of SIRS on day 1 post-injury was 68% according to Table 2, which was different to that reported in Table 1 (92%). Figure 1 was in agreement with Table 2, while the data in the text were in agreement with Table 1. These inconsistencies cast some doubt on the data presented.

Address for reprints: GV Bochicchio, Department of Surgery, R Adams Cowley Shock Trauma Center, 22 South Greene Street, Baltimore, MD 21201, USA. Email: gbochicchio@umm.edu

PATHOGENESIS

Nitric oxide involvement in the hemodynamic response to fluid resuscitation in endotoxic shock in rats

Losser MR, Forget AP, Payen D.
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The initial phase of septic shock is characterized by a low blood pressure and reduced systemic blood flow. Initial care, consisting of massive fluid challenge, often fails to improve arterial hypotension leading to catecholamine administration. This experimental study investigated the role of nitric oxide (NO) in the fluid loading-induced modifications of the heart and vascular functions in endotoxemic rats. Rapid fluid loading improved systolic cardiac function and induced vasodilation. Pretreatment with an NO synthase inhibitor (L-N^G-nitroarginine) fully blocked the vasodilation induced by fluid loading in control animals and only partially in lipopolysaccharide (LPS)-treated animals, probably due to presence of LPS-induced endothelial dysfunction. This study demonstrated that fluid loading in septic animals is responsible for vasodilatory state related to the NO pathway.

It has recently been shown that the initial phase of septic shock is characterized by low blood pressure and decreased systemic blood flow [1]. Fluid challenge during this initial phase often fails to improve arterial hypotension, necessitating catecholamine administration. The exact mechanisms of fluid failure have not been extensively studied. In this experimental study, the authors investigated the effects of fluid resuscitation on heart and vascular functions in rats with endotoxic shock, in particular, the involvement of the nitric oxide (NO) pathway.

Under general anesthesia, animals were monitored with arterial and intravenous catheters, and placement of a Doppler transducer around the ascending aorta to allow measurements of aortic blood flow velocity (V_{Ao}), maximal acceleration (G_{max}), and arterial conductance (C_{Art}). Endotoxic shock was induced by intravenous injection of 5 mg/kg lipopolysaccharide (LPS). After 165 min (T165), mean arterial pressure (MAP) significantly decreased in LPS animals (a reduction of 30% compared with control animals), as did V_{Ao} (a reduction of 20%), but there were no changes in G_{max} and C_{Art}. At that time, half of animals were intravenously injected with the NO synthase (NOS) inhibitor L-N^G-nitroarginine (LNA). This caused an increase in MAP with decreased V_{Ao}, G_{max}, and C_{Art} in control and LPS-treated animals. Fluid challenge with 15 mL/kg hydroxyethyl starch was performed after 15 min (T180). In the absence of LNA, fluid loading increased MAP, V_{Ao}, G_{max}, and decreased C_{Art} in control animals and in rats that received LPS. In LNA-treated animals, fluid loading increased MAP and V_{Ao} in control and LPS-treated groups similarly, without any effects on G_{max}. C_{Art} increased in control animals after fluid challenge but less so in the LPS-treated group. This could be explained by presence of LPS-induced endothelial dysfunction. L-arginine infusion, administered in order to reverse LNA effects, induced a marked increase in C_{Art} in both group, with this increase being more pronounced in LPS-treated animals due to L-arginine induced hypotension. V_{Ao} and G_{max} were not modified by L-arginine infusion.

This study demonstrated that LPS caused hypokinetic shock with low blood pressure, low systemic blood flow, and unchanged conductance in the absence of any resuscitation. In control and LPS-treated animals, rapid fluid loading improved systolic cardiac function (increased G_{max}) and induced vasodilation (decreased C_{Art}). The LNA-induced impairment of systolic left ventricular performance did not improve after fluid loading in either control or LPS-treated animals. LNA completely blocked the vasodilation induced by fluid loading in control animals and only partially in LPS animals. In the latter, the presence of endothelial dysfunction may explain the disparity, and the different roles of the NO pathway in the heart and in the regulation of vascular tone.

These results have to be interpreted with care since the effects were demonstrated during the early phase of sepsis (3 h after LPS induction). This short delay may not have involved inducible NOS-mediated production of NO. Nevertheless, this study demonstrated that fluid loading in septic animals is responsible for the vasodilatory state, and

that the NO pathway is involved in this function. Further investigations are required to define the exact mechanisms of the fluid challenge-mediated vasodilatory effect.

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Address for reprints: MR Losser, Laboratoire du Département d'Anesthésie–Réanimation, Centre Hospitalier Universitaire Lariboisière, Assistance Publique–Hôpitaux de Paris, Université Paris 7, Paris, France.

Interleukin-1-dependent sequential chemokine expression and inflammatory cell infiltration in ischemia-reperfusion injury

Furuichi K, Wada T, Iwata Y et al.
Crit Care Med 2006;**34**:2447–55.

Inflammation, in particular the presence of interleukin-1, is implicated in the development of tissue damage during ischemia–reperfusion injury.

Ischemia–reperfusion injury is a complex process occurring in various conditions such as organ transplantation, surgical procedures including vessel clamping, or recovery after cardiac arrest. Ischemia–reperfusion injury leads to organ damage, but the pathophysiological mechanisms involved remain to be elucidated. The severity of organ injury depends on the length of the ischemic period, but, interestingly, most lesions occur after reperfusion. Histological analyses demonstrate that inflammatory processes are involved in the tissue destruction. Thus, the current authors investigated the role of one of the key elements of inflammation, interleukin-1 (IL-1). The IL-1 family comprises IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1RA). These bind to the same receptor; however, IL-1RA does not induce intracellular signals. IL-1 plays a key role in the activation of the inflammatory cascade, stimulating numerous types of cells to synthesize tumor necrosis factor, interferon, IL-8, monocyte chemoattractant protein-1 (MCP-1), and IL-1 itself. The authors investigated the role of IL-1α, IL-1β, and IL-1RA in ischemia–reperfusion injury using IL-1α-, IL-1β-, and IL-1RA-deficient mice.

Wild-type, IL-1α-deficient, IL-1β-deficient, and IL-1RA-deficient mice were submitted to 45 min of renal ischemia (clamping) under anesthesia. Sham animals that underwent operations, but no clamping, were also included. Renal tissues and blood samples were obtained at baseline and at 24 h, 48 h, and 96 h post-injury. In addition to classical histological examination, apoptosis was assessed by the dUTP-biotin nick-end labeling method, and immunohistochemical studies were performed to assess local production of the chemokines MCP-1 and macrophage inflammatory protein-1α. Serum levels of these chemokines were also evaluated. Finally, proximal tubular cell cultures were stimulated by IL-1β and/or H_2O_2, and chemokine expression evaluated.

IL-1β levels increased in the serum of wild-type and IL-1RA-deficient mice, but not in IL-1α- or IL-1β-deficient mice. Similarly, chemokine levels were lower in IL-1α- or IL-1β-deficient mice compared with wild-type and IL-1RA-deficient mice. Renal tissue destruction was observed in wild-type and IL-1RA-deficient mice but not in IL-1α- or IL-1β-deficient mice, and the lesions were more severe in IL-1RA than in wild-type mice. Neutrophilic infiltration in the kidney correlated both with acute tubular necrosis and with local expression of chemokines. Apopotosis was lower in IL-1α- or IL-1β-deficient mice, but was increased in IL-1RA-deficient mice. Finally, while exposure of proximal tubular cells to IL-1β alone did not lead to necrosis or apoptosis, exposure to IL-1β in combination with H_2O_2 led to a much more pronounced increase in necrotic and apoptotic cells, compared with H_2O_2 alone.

This study illustrates the role of inflammation, specifically IL-1, in the pathophysiology of ischemia–reperfusion injury.

Address for reprints: K Furuichi, Department of Gastroenterology and Nephrology and Division of Blood Purification, Kanazawa University, Kanazawa, Japan.

Mechanisms of mortality in early and late sepsis

Xiao H, Siddiqui J, Remick DG.
Infect Immun 2006;**74**:5227–35.

In the mouse model of sepsis employed in this study, early mortality was associated with an excessive inflammatory response while later deaths were characterized by a suppressed inflammatory response. The paper also presents data describing the effects of surgery, emphasizing its key role in the control of the source of sepsis.

Although the initial pathophysiological findings in sepsis usually reflect a hyperactive inflammatory response, the later stages are usually associated with signs of decreased immune response, as reflected by the high rate of nosocomial infections. The authors used a mouse model of abscess to investigate the immune response in early and late phases of sepsis and correlated these findings with the outcome of the animals.

A standardized procedure of cecal ligation and perforation was performed in 121 mice. Fluid resuscitation (with a standardized amount) and antibiotics were administered. At day 4, surviving mice were randomized into two groups, one receiving antibiotics alone (control animals) and the second undergoing surgery (resection of necrotic cecum plus washing of the peritoneal cavity) in addition to

antibiotics for a further 2 days. Temperature and body weight were determined on a daily basis, and blood was obtained at specific times for measurement of cytokine levels. Mice were sacrificed at predefined intervals and the size of intraperitoneal abscess was measured. Peritoneal fluid was cultured, and peritoneal cytokine levels measured. In addition, peritoneal cells were stimulated *in vitro* by endotoxin or via toll-like receptor 2, and cytokine expression was measured.

Twenty percent of the mice died prior to day 4. Mice that underwent cecal resection at day 4 had a lower mortality rate (5%) between days 4 and 21 than mice allocated to antibiotics alone (33%). Cecal resection dramatically reduced the size of the peritoneal abscess, which was almost undetectable in this group. In the control mice, abscess size was 4 mm^3 on day 11, but decreased to 1 mm^3 on day 21. This may either represent a spontaneous resorbtion of the abscess or an association between abscess size and outcome, since mice continued to die in the control group between day 11 and day 21 (suggesting that only mice with a small abscess size were able to survive).

The evolution of mouse weight was also interesting. In mice surviving beyond day 4, weight decreased initially in all animals; it further decreased in the 24 h after surgery (probably related to the negative metabolic impact of the resection procedure) but thereafter, operated animals had a more rapid weight recovery than non-operated animals. Mice that died in the acute phase (prior to day 4) gained weight continuously before death, reflecting a generalized increase in permeability and edema, while animals dying at later stages lost weight in the 5 days preceding death, reflecting starvation.

After a transient neutropenia, the numbers of white blood cells increased markedly in all animals; this increase was reversed by surgery. Mice that were found to have a large abscess size at sacrifice had higher neutrophil counts. Non-surviving mice had higher neutrophil counts than surviving mice, regardless of whether resection surgery had been performed. Bacterial growth in the peritoneal cavity was higher in non-operated mice than in animals that underwent cecal resection; furthermore, bacterial growth was higher in non-surviving compared with surviving animals.

Plasma interleukin-6 (IL-6) levels were higher in animals dying within the first 4 days than in those that survived, but did not differ between surviving and non-surviving mice after day 4. More macrophages were recovered from the peritoneal cavity of moribund animals compared with healthy animals, but stimulation of these peritoneal cells resulted in a higher release of IL-6 from cells of healthy animals than moribund mice.

Collectively, these data suggest that animals dying early in the course of infection had an enhanced inflammatory response, while animals dying at later stages were immuno-incompetent and were not able to cure the abdominal infection. These data also emphasize the beneficial role of surgery in controlling the source of infection.

Address for reprints: DG Remick, Department of Pathology, University of Michigan, 2210 Medical Science I Building, 1301 Catherine Road, Ann Arbor, MI 48109-0602, USA. Email: remickd@umich.edu

RESEARCH

Modulation of the triggering receptor expressed on myeloid cells-1 pathway during pneumonia in rats

Gibot S, Alauzet C, Massin F et al.
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In a rat model of lung pneumonia, pretreatment of animals with a synthetic peptide antagonizing the cell surface molecule, triggering receptor expressed on myeloid cells-1, blunted the inflammatory response, limited hemodynamic and metabolic alterations, and improved the disease outcome.

Triggering receptor expressed on myeloid cells-1 (TREM-1) plays a role in the amplification of the inflammatory response in sepsis. It is a cell-surface molecule belonging to the immunoglobulin superfamily of receptors and is upregulated on neutrophils and monocytes in the presence of Gram-positive and Gram-negative bacteria or fungi, but not in aseptic inflammatory conditions. In addition, TREM-1 amplifies synthesis of proinflammatory cytokines in the presence of endotoxin. A synthetic peptide (LP17) that mimics interspecies-conserved domains but is deprived of proinflammatory properties was tested in a rat model of *Pseudomonas aeruginosa*-induced pneumonia. It was anticipated that LP17 might limit lung damage and death.

Rats were randomly allocated to receive saline instillation in the lung (sham), *P aeruginosa* instillation (control), or LP17 instillation followed 5 min later by *P aeruginosa* (LP17). Mechanical ventilation was initiated 18 h after instillations with a tidal volume of 7–8 mL/kg and a fraction of inspired oxygen (FiO_2) of one. Arterial blood pressure and blood gas, bronchoalveolar lavage (BAL), histological examination, and survival rate were assessed.

Arterial blood pressure decreased in the control group while it was preserved in the LP17 group. Lactic acidosis and arterial hypoxemia were less severe in the LP17 group than in control group. The BAL fluid revealed a marked neutrophilic infiltration that was not prevented by prior administration of LP17.

The elevation of tumor necrosis factor, interleukin-1β (IL-1β), and IL-6 that occurred in the blood and lungs of the control animals was reduced by LP17. Similarly, LP17 blunted the activation of coagulation, as evaluated by D-dimer and thrombin–antithrombin complex concentrations in the blood and BAL fluid.

At 24 h after bacterial instillation, histological examination of the lungs disclosed intra-alveolar hemorrhage, protein precipitation, leukocyte infiltration into the alveoli, and thickening of the perivascular space. These lesions were attenuated by LP17. The 1-week survival rate was 100% in the sham group, 14% in the control group, and 43% in LP17-pretreated animals.

Several limitations of this study should be recognized. No antibiotics were used and it is possible that the observed protective mechanisms may be of less significance in circumstances when they are administered. Secondly, fluid administration was minimal and this may also have influenced the outcome of the animals. Finally, LP17 was administered as a pretreatment, and many interventions given early have failed to confirm efficacy when administered as a delayed treatment. Thus, although promising, these results need to be confirmed in more clinically relevant models.

Address for reprints: S Gibot, Service de Reanimation Medicale, Hôpital Central, 54035 Nancy, France. Email: s.gibot@chu-nancy.fr

A proteomic analysis of liver mitochondria during acute endotoxemia

Crouser ED, Julian MW, Huff JE et al.
Intensive Care Med 2006;**32**:1252–62.

The authors of this study performed a proteomic analysis of mitochondria in order to identify the mitochondrial functions affected by sepsis. They demonstrated that endotoxin induces changes in the expression of several mitochondrial proteins.

The pathophysiology of sepsis-induced multiple organ dysfunction is likely to be multifactorial. In addition to hemodynamic alterations including global, regional, and microvascular blood flow alterations, mitochondrial dysfunction may play a role in the development and progression of organ dysfunction. Morphological alterations of mitochondria have been described but definitive evidence linking these alterations to organ dysfunction is lacking.

Mitochondrial function is not restricted to oxidative phosphorylation, it also participates in calcium regulation, lipid and protein metabolism, and apoptosis. In this paper, Crouser et al. investigated the effects of sepsis on mitochondrial proteomics in order to better understand which mitochondrial functions may be affected by sepsis.

Adult, male cats were anesthetized and administered intravenous endotoxin or saline vehicle. Standard resuscitation procedures were provided in both groups. Liver oxygenation was evaluated by *in vivo* reflectance spectrophotometry. Mitochondria were isolated from excised livers and cellular proteins were prepared for proteomic analyses on two-dimensional gel electrophoresis. Of note, all samples in each group were mixed to avoid inter-individual variability.

There was no alteration in hemodynamics or acid–base balance. More than 500 protein spots were identified, but some of the spots could not be identified due to limitation in the resolution of the gel. Lipopolysaccharide induced different expression characteristics of approximately 8% of protein spots. There was a significant reduction in the expression of hydroxymethylglutaryl-coenzyme A synthase and heat shock protein 70 (HSP70). Conversely, levels of two enzymes critical for the urea cycle (HSP60 and manganese superoxide dismutase) were increased.

These data suggest that there is differential mitochondrial protein expression in sepsis, with an increase in enzymes involved in the urea cycle, a decrease in an enzyme implicated in ketogenesis and cholesterol biosynthesis, and alterations in levels of chaperone proteins implicated in repair after oxidant stress. The functional implications of these changes remain to be elucidated.

Address for reprints: ED Crouser, Division of Pulmonary, Critical Care and Sleep Medicine, Dorothy M Davis Heart and Lung Research Institute, The Ohio State University, 473 West 12 Avenue, Columbus, OH 43210-1252, USA. Email: elliott.crouser@osumc.edu

THERAPEUTICS RESEARCH

Attenuation of capillary leakage by hydroxyethyl starch (130/0.42) in a porcine model of septic shock

Marx G, Pedder S, Smith L et al.
Crit Care Med 2006;**34**:3005–10.

The authors of this study compared a new version of hydroxyethyl starch (130/0.42) with an older version of this synthetic colloid (200/0.5) in a porcine model of sepsis. Their results showed that administration of the new version was able to reduce capillary leakage, as measured by albumin escape. This new colloid may represent a useful tool in volume resuscitation of patients with septic shock.

Central to the pathophysiology of sepsis is the dysfunction of blood vessels leading to plasma leakage. This serves both to

decrease circulating blood volume, which can decrease oxygen delivery, and to increase tissue edema, which can lead to organ dysfunction. Administration of colloid, as opposed to crystalloid fluids, has been shown to help reduce tissue edema formation while providing adequate intravascular volume expansion. Hydroxyethyl starch (HES) is a synthetic colloid solution that has been used in models of sepsis – with similar findings. The authors of this study evaluated a newer version of HES in a porcine model of sepsis.

In this study, the investigators compared administration of 200/0.5 HES with the newer 130/0.42 HES, which is characterized by a smaller molecular weight. The pigs were made septic with peritonitis and HES was used for volume resuscitation. Capillary leakage was monitored by measuring the albumin escape rate. The results showed that administration of 130/0.42 HES was associated with a lower capillary leak rate compared with the 200/0.50 formulation. The authors do not know the mechanism underlying this improved function but they hypothesize that drug pharmacokinetics may play a role.

This study demonstrates that further improvements in the effectiveness of synthetic colloids (compared with existing solutions) for sepsis are possible. In addition to these results, the authors point out that the newer HES solution is characterized by less alteration of coagulation in the patients who receive it.

Address for reprints: G Marx, Department of Anaesthesiology and Intensive Care, Friedrich Schiller University, Erlanger Allee 101, 07740 Jena, Germany. Email: gernot.marx@med.uni-jena.de

Effect of recombinant activated protein C and low-dose heparin on neutrophil-endothelial cell interactions in septic shock

Kirschenbaum LA, Lopez WC, Ohrum P et al.
Crit Care Med 2006;**34**:2207–12.

Using an *in vitro* model of neutrophil rolling and adhesion, the authors of this study demonstrated that drotrecogin alfa (activated) (recombinant human activated protein C) decreased sepsis-induced neutrophil adherence to endothelium and neutrophil aggregation, which is thought to be associated with a reduction in platelet–neutrophil aggregation. These effects may explain how drotrecogin alfa (activated) plays a role in preservation of the microcirculation in septic conditions.

Septic shock is characterized by inflammation of the endothelium and activation of coagulation mechanisms, which both contribute to impaired microvascular blood flow. Drotrecogin alfa (activated) (recombinant human activated protein C) has been shown to exert anti-inflammatory effects, in particular, by attenuating leukocyte adhesion to endothelial cells. Low-dose (LD) heparin has also been shown to have a protective role in sepsis through its anti-inflammatory properties and attenuation of cell adhesion. This study compared the effects of drotrecogin alfa (activated) and LD heparin on sepsis-related neutrophil–endothelial and platelet–endothelial interactions in an *in vitro* model of capillary blood flow.

The study was divided into several sets of experiments. Serum or plasma was taken from 21 septic shock patients and six healthy volunteers. The first experiment used neutrophils and platelets stimulated with septic plasma (containing clotting factors) or septic serum (depleted of clotting factors), with or without drotrecogin alfa (activated). By analyzing the effects of both plasma and serum, it was possible to separate the anti-inflammatory effects of drotrecogin alfa (activated) from those related to its anticoagulant effect. Drotrecogin alfa (activated) was demonstrated to significantly decrease neutrophil adhesion to endothelial cells and leukoaggregation, while neutrophil rolling velocity was increased. Platelet–neutrophil aggregates in septic plasma were decreased by drotrecogin alfa (activated). In a second set of experiments, cells were incubated with or without LD heparin; this did not significantly affect sepsis-related neutrophil aggregation, adherence, rolling velocity, or platelet–neutrophil aggregation. In the third set of experiments, addition of LD heparin to the drotrecogin alfa (activated) in septic plasma attenuated the effects compared with drotrecogin alfa (activated) alone on neutrophil adherence and aggregation and rolling velocity. No effect on the binding of activated platelets to neutrophils was observed.

Since similar changes were observed when using septic serum and plasma, it is not possible to separate the anti-inflammatory effects of drotrecogin alfa (activated) from its anticoagulant actions. These results are consistent with several studies demonstrating a link between inflammation and coagulation via the protease activated receptor 1. In this study, drotrecogin alfa (activated) interfered with neutrophil–endothelial cell interactions via reduction of platelet–neurophil aggregation. The absence of any beneficial effect of LD heparin when in combination with drotrecogin alfa (activated) is consistent with results from the PROWESS (Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis) study [1], in which heparin administration interfered with the beneficial effects of drotrecogin alfa (activated). The mechanisms underlying the response to LD heparin remain unclear and, as the authors suggest, additional work is required, in particular to evaluate the effect of low-molecular-weight heparin. One limitation to this study is the use of an *in vitro* model where

different shear stress conditions were not simulated; this is likely to play an important role in cell–cell interactions. The absence of erythrocytes may also affect the results.

This study presents a novel area of research with regard to the properties of drotrecogin alfa (activated), demonstrating its potential role in preserving microcirculation by attenuating neutrophil–endothelial cell interactions in septic conditions.

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Address for reprints: LA Kirschenbaum, Department of Medicine, New York Medical College, Valhalla, NY, USA.

Preclinical trial of L-arginine monotherapy alone or with *N*-acetylcysteine in septic shock

Kalil AC, Sevransky JE, Myers DE et al.
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L-arginine is the substrate for nitric oxide (NO), which is an endogenous mediator that is associated with sepsis-induced shock. In this study, when parenterally infused in a canine septic shock model, L-arginine had harmful effects, including an increased mortality rate in septic animals with worsened shock, hyperchloremic acidosis, and organ failure. This toxicity appears to be related to increased production of NO. Thus, L-arginine should be avoided in septic patients.

L-arginine is a non-essential amino acid that is proposed to be conditionally essential in septic conditions. It is known to have a number of diverse activities; however, the impact of these on the outcome of sepsis is largely unknown. Consequently, its administration in sepsis is controversial. This study tested the therapeutic efficacy of parenteral L-arginine administration in a well-characterized canine model of septic shock.

Sepsis was induced under general anesthesia by intraperitoneal placement of *Escherichia coli*, mimicking peritonitis. Parenteral L-arginine was continuously infused at a rate of 10 or 100 mg/kg/h for 36 h, equivalent respectively to 1.5- to 15-times the dose routinely used in patient total parenteral nutrition. L-arginine was infused without or with infusion of *N*-acetyl-cysteine (20 mg/kg/h for 36 h), which was given as an antioxidant agent, thus increasing nitric oxide (NO) bioavailability and preventing peroxynitrite formation. The septic model used is a resuscitated model in which antibiotics and fluid challenge are administered throughout the analysis.

In low- and high-dose L-arginine-treated animals, the mortality rate significantly increased compared with control animals. *N*-acetylcysteine infusion had no effect on the increased mortality rate. L-arginine infusion lowered mean arterial pressure, worsening shock, irrespective of the presence of *N*-acetylcysteine. L-arginine was well absorbed with a significant increase in arginine and ornithine plasma concentrations observed in L-arginine-treated animals compared with the control group. Nitrate/nitrite levels increased significantly after 8 h compared with control animals, as did serum markers of renal function (blood urea nitrogen/creatinine ratios), while serum liver enzymes did not increase significantly. Animals treated with L-arginine alone or in combination with *N*-acetylcysteine had greater decreases in mean arterial pH and bicarbonate levels.

From these results, the authors concluded that L-arginine administration at supradietary doses is harmful in this septic shock model. Furthermore, addition of *N*-acetylcysteine could not inhibit or reduce these harmful effects of L-arginine. The authors could not infer any information regarding the precise mechanisms of L-arginine toxicity from this study. It may be related to increased production of NO, but this needs further study. Regardless, these results are in accordance with the recommendation of the Canadian Clinical Practice Guidelines for nutrition support in critical illness, which state that diets supplemented with L-arginine should not to be used in critically ill patients [1].

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Address for reprints: A Kalil, 985400 University of Nebraska Medical Center, Omaha, NE 68198, USA. Email: akalil@unmc.edu

CLINICAL TRIALS

Vasopressin or norepinephrine in early hyperdynamic septic shock: a randomized clinical trial

Lauzier F, Lévy B, Lamarre P et al.
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Since vasopressin levels decrease 36 h after onset of septic shock, administration of exogenous arginine–vasopressin (AVP) is considered to be important. AVP is mainly used as a rescue therapy in refractory septic shock. In this study, AVP alone failed to maintain mean arterial pressure at >70 mmHg when administered during the early phase of septic shock. However, AVP improved sequential organ failure assessment score and had no significant adverse effects on gastrointestinal, renal, or liver functions.

Vasopressin deficiency has been shown to occur 36 h after the onset of septic shock [1]. Therefore, it appears relevant to administer exogenous arginine–vasopressin (AVP) to septic shock patients. There are multiple reports in the literature of studies on the use of AVP in septic shock; however, these

were uncontrolled or retrospective. In this study, the authors prospectively evaluated the effects of AVP in the early management (in the first 12 h) of hyperdynamic shock.

Twenty-three patients suffering from septic shock were randomized to receive AVP at a dose of 0.04–0.20 U/min (n=13), or norepinephrine (NE) at a dose of 0.1–2.8 μg/kg/min (n=10). Patient characteristics were similar at baseline with the exception of higher bilirubin levels in the AVP group. When the maximal dosage of the experimental drug was reached, administration of the other drug was allowed as rescue therapy if the mean arterial pressure (MAP) was <70 mmHg. AVP and NE similarly increased MAP over 48 h. AVP decreased cardiac index by decreasing heart rate. The NE dose was lower in the AVP group at the end of the study compared with baseline, but 85% of AVP-infused patients received NE at some point (due to a MAP of <70 mmHg despite maximal dose of AVP of 0.2 U/min). No patients in the NE group received rescue AVP therapy. AVP was shown to improve sequential organ failure assessment (SOFA) score and creatinine clearance compared with NE. During the course of the study, the change in bilirubin levels from baseline did not differ between the AVP and NE groups. No difference between NE and AVP was demonstrated with respect to platelet count and gastric-arterial pCO_2 difference, even though AVP increased systemic vascular resistance. The mortality rate was similar in both groups (three died during the study from refractory shock). One patient in each group suffered from an acute coronary syndrome.

In this study, the authors demonstrated that high-dose AVP alone did not maintain MAP above 70 mmHg in the early resuscitation phase of hyperdynamic septic shock patients. Patients enrolled in this study were in septic shock for <12 h before AVP depletion occurred. AVP levels were not measured throughout the study; however, even if it had been done, this would not have taken into account the decreased sensitivity to AVP. This study showed an improvement of SOFA score and renal function without adverse effects on gastric-arterial pCO_2 difference or liver function. Moreover, AVP decreased NE exposure. Two limitations of this study are that it was performed on a small number of patients, and clinicians were not blinded to the drug used.

This is the first study to evaluate AVP as primary therapy of septic shock rather than as a rescue therapy. These results indicate a novel use for AVP early in septic shock patients, and the authors suggest that a larger, controlled, double-blind, randomized trial should be undertaken.

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Address for reprints: O Lesur, Centre Hospitalier Universitaire de Sherbrooke, Soins Intensifs Médicaux et Chirurgicaux, Centre de Recherche Clinique, 12ème Avenue Nord, 3001, J1H 5N4 Sherbrooke, QC, Canada. Email: olivier.lesur@usherbrooke.ca

DIAGNOSIS AND ASSESSMENT

Renal failure in septic shock: predictive value of Doppler-based renal arterial resistive index

Lerolle N, Guérot E, Faisy C et al.
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Acute renal failure (ARF) frequently occurs during septic shock. Renal arterial resistive index (RI) can be assessed using Doppler ultrasonography and has been shown to be correlated with renal vascular resistance. The authors of this study demonstrated that the detection of increased RI at day 1 of septic shock is helpful in identifying patients who will develop ARF.

During septic shock, hemodynamic failure and the induction of an inflammatory cascade are responsible for acute tubular necrosis, leading to acute renal failure (ARF). ARF is often diagnosed at a late stage, on the basis of functional markers such as oliguria and serum creatinine concentration. In this study, the authors assessed renal arterial resistive index (RI), which is correlated to renal vascular resistance; hypothesizing that RI could be an early marker of renal dysfunction in septic patients.

Thirty-seven medical intensive care unit septic shock patients without chronic renal failure were enrolled in this study. A Doppler-ultrasonography-based calculation of RI was performed at day 1 in patients in whom hemodynamic stability for >1 h (adequate fluid challenge and absence of change of catecholamine infusion) was obtained. ARF was diagnosed according to the RIFLE (risk of renal dysfunction, injury to the kidney, failure of kidney function, loss of kidney function, and end-stage kidney disease) criteria [1] at day 5. In the 18 patients who were diagnosed as having ARF at day 5, RI was higher compared with the 17 patients who did not have ARF. This was associated with higher Simplified Acute Physiology Score II and arterial lactate concentration. In this study, the authors specified that RI was determined during catecholamine infusion at a stable rate, and in the absence of diuretic use (even if diuretics did not affect RIFLE classification). The receiver operating characteristic (ROC) curve for RI as a predictor of renal dysfunction at day 5 showed that an RI of >0.74 (normal range 0.5–0.71) had a positive likelihood ratio for severe renal dysfunction of 3.3. RI was similar in patients treated with dopamine or epinephrine, and no dose effect of either drug was demonstrated. Finally, the authors showed that RI was inversely correlated with mean arterial pressure (MAP), but not with lactate concentration.

In this well-designed study, the authors verified that RI was not influenced by the dose of the catecholamine used. This is an important point, which was confirmed by the inverse correlation with MAP. Indeed, if RI were determined by vasopressor, a positive correlation would have been found. These study results are in accordance with previous experimental and clinical studies. One remaining limitation of this study is the actual significance of RI: does it indicate renal failure or just the risk of it? Another limitation is related to the delay, with RI assessment performed one day after the occurrence of septic shock. The determination of the beginning of septic shock is a universal problem. Septic shock is diagnosed when the hemodynamic situation dictates the need for catecholamines, as defined by Bone's criteria [2]. At that time, ARF is patent. However, for the time being, there is no early marker indicating the shift from severe sepsis to septic shock.

In spite of these limitations, Doppler-based RI performed 1 day after septic shock appears to be helpful in identifying patients who will develop ARF. Further studies are required to confirm this.

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Address for reprints: N Lerolle, Université Paris-Descartes, Faculté de Médecine, Assistance Publique–Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Service de Réanimation Médicale, 20, Rue Leblanc, 75908 Paris Cedex 15, France. Email: nicolas.lerolle@egp.aphp.fr

Activated partial thromboplastin time waveform analysis: a new tool to detect infection?

Chopin N, Floccard B, Sobas F et al.
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The authors of this study found that the activated partial thromboplastin time waveform, which is altered in sepsis, can be used to identify patients who have, or are at risk of developing, severe sepsis or septic shock.

Calcium-dependent formation of complexes between very low-density lipoprotein and C-reactive protein (CRP) can induce abnormalities in the optical transmission waveform during measurements of the activated partial thromboplastin time (aPTT) using specific photometric analyzers. These abnormalities, characterized by a biphasic waveform, are not related to an alteration in the coagulation properties determined by aPTT, but are associated with sepsis. They usually last 2–3 days and precede the full onset of disseminated intravascular coagulation and sepsis. The authors performed this study to assess the predictive value of an abnormal aPTT waveform for the diagnosis and prognosis of sepsis.

All consecutive patients admitted to a single intensive care unit over a 9-month period were investigated. On admission and daily for 28 days, systemic inflammatory response syndrome (SIRS), sepsis, severe sepsis, and septic shock criteria were evaluated and categorized as an event (for example, septic shock occurring on day 21 in a patient initially admitted for drug intoxication would be classified as an event, as would that in a patient initially admitted for septic shock). The most severe event was recorded when these were related (e.g. SIRS leading to septic shock), but multiple independent events could occur in the same patient (i.e. severe sepsis cured but later suffer a new episode of septic shock related to nosocomial infection). Sequential Organ Failure Assessment score was calculated at days 1, 2, and 3; procalcitonin (PCT), CRP, lactate levels, and aPTT waveform were measured at days 1 and 3. A biphasic waveform was defined as an initial slope (slope_1) higher than three standard deviations of the slope of healthy volunteers, and corresponded to a slope larger than –0.25% transmission/sec (% T/s). Survival at day 28 was also assessed.

A total of 355 patients were admitted to the ICU during the study period; of these, 187 presented at least one event, with the total number of events being 217 as some subjects had several independent events. In patients who were classed as having an event, a biphasic aPTT waveform was observed in 53% upon admission and in the remainder of the patients (47%) on subsequent days. The aPTT slope was steeper in patients with severe sepsis and septic shock than in patients with SIRS and sepsis. Furthermore, the aPTT slope was steeper in sepsis nonsurvivors compared with septic survivors on admission, and although this slope tended to improve over time, it remained steeper in non-survivors than in survivors at all times. The receiver operating characteristic curve area was similar for aPTT waveform, PCT, and CRP measurements at day 1 for predicting severe sepsis and septic shock. Outcome prediction was similar for aPTT waveform, PCT, and lactate measurements on day 3, while CRP performed less adequately.

These results show that aPTT waveform analysis can be used to identify patients who are at risk of developing severe sepsis and septic shock and who are at higher risk of death. Whether this information really helps the clinician (compared with measurements of PCT, CRP, and lactate) remains to be elucidated.

An important limitation of the study is the occasionally long delay between biological measurements and the onset of the event. Another limitation is that the results of PCT, CRP, and lactate tests were known by the physicians when treating the

patients, and this may have affected the prognostic value of these variables (i.e. a diagnosis of sepsis may have been more rigorously assessed when one or more of these were altered).

Address for reprints: N Chopin, Service de Réanimation Chirurgicale, Hospices Civils de Lyon, Hôpital Edouard Herriot, Lyon, France.

Mixed venous oxygen saturation cannot be estimated by central venous oxygen saturation in septic shock

Varpula M, Karlsson S, Ruokonen E et al.
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The authors of this study used paired samples of blood from the superior vena cava and pulmonary artery to assess the accuracy of using central venous lines to measure mixed-venous oxygen saturation (SvO_2) in patients with septic shock. Their results show that while there is some correlation the values vary significantly and therefore central venous O_2 may not be an accurate substitute for true SvO_2.

New models for hemodynamic resuscitation in sepsis call for the assessment of cardiac output by analyzing blood gas from a sample obtained by a central venous line in the neck or central mixed-venous O_2 ($ScvO_2$). It is thought that the values will be close to the true mixed-venous O_2 (SvO_2) level that is sampled from the pulmonary artery with a Swan-Ganz catheter. The authors of this study sought to determine whether $ScvO_2$ was in fact an accurate surrogate for using SvO_2 as a marker of cardiac output in septic shock.

The authors obtained paired samples of blood from 16 patients with septic shock and also obtained a measurement of cardiac output via thermodilution for 24 h. It is interesting to note that the $ScvO_2$ was obtained from the side port of the introducer, typically well within the superior vena cava. Analysis of the data showed a statistically significant correlation of the paired samples (in general, they moved up or down together); however, 45% of the time they did not correlate well. They also showed poor agreement, with significant differences in the values consistent throughout all measurements and the difference between the two measurements became more pronounced at either very high or very low values. Mean SvO_2 was consistently lower than the mean $ScvO_2$.

This study demonstrates that if accurate assessment of a patient's cardiac output is required over time, the use of an $ScvO_2$ may not be appropriate. Using the measurement for clinical purposes or to assess outcomes was not considered. It should be noted that this study was small, involving only 16 patients; furthermore, these subjects were all suffering from septic shock, which is a condition that can alter oxygen extraction and thus affect SvO_2 independently of cardiac output.

Address for reprints: M Varpula, Helsinki University Hospital, POB 340, 00029 HUS, Helsinki, Finland. Email: marjut.varpula@hus.fi

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