Review

Microvascular resuscitation as a therapeutic goal in severe sepsis

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Abstract

Sepsis causes microvascular dysfunction. Increased heterogeneity of capillary blood flow results in local tissue hypoxia, which can cause local tissue inflammation, impaired oxygen extraction, and, ultimately, organ dysfunction. Microvascular dysfunction is clinically relevant because it is a marker for mortality: it improves rapidly in survivors of sepsis but fails to improve in nonsurvivors. This, along with the fact that resuscitation of mean arterial pressure and cardiac output alone fails to improve microvascular function, means that microvascular resuscitation is therefore a therapeutic goal. In animal studies of sepsis, volume resuscitation improves microvascular permeability and tissue oxygenation, and leads to improved organ function, including a reduction in myocardial dysfunction. Microvascular resuscitation strategies include hemodynamic resuscitation using the linked combination of volume resuscitation, judicious vasopressor use, and inotropes and vasodilators. Alternative vasoactive agents, such as vasopressin, may improve microcirculatory function to a greater degree than conventional vasopressors. Successful modulation of inflammation has a positive impact on endothelial function. Finally, targeted treatment of the endothelium, using activated protein C, also improves microvascular function and ultimately increases survival. Thus, attention must be paid to the microcirculation in patients with sepsis, and therapeutic strategies should be employed to resuscitate the microcirculation in order to avoid organ dysfunction and to reduce mortality.

Introduction

Impaired microvascular function is increasingly recognized as a key characteristic contributing to organ dysfunction and death in patients with sepsis. In fact, impaired oxygen extraction in patients who have sepsis was recognized more than 40 years ago, although it is only more recently that there has been consensus among investigators that microvascular dysfunction is a central feature of sepsis, accounting for many characteristics of the pathogenesis of septic organ dysfunction [1-5]. Indeed, a key observation is that therapeutic modalities that may improve microvascular function are also associated with decreased organ dysfunction and improved outcome in patients with sepsis [6-8]. Furthermore, since normal endothelial function underlies normal microvascular function, there is an increasing interest in endothelial function during sepsis [9-12]. It is therefore vital to understand as much as possible about the microcirculation and endothelium in sepsis in order to identify therapeutic strategies for resuscitating the microcirculation and thus improving outcome.

Microvascular dysfunction in sepsis

The clinical observations of cyanosis with mottled skin, and evidence of tissue hypoxia (e.g. elevated lactate levels) despite high cardiac output, are common and long-recognized in patients with septic shock. Taken together with reports of abnormal sublingual perfusion in septic patients [13-15], these simple observations suggest that microvascular dysfunction occurs and leads to impaired tissue oxygen transport. Recently, microvascular function has been examined more vigorously in experimental models of sepsis where increased heterogeneity of microvascular perfusion is a hallmark of sepsis. Ellis and colleagues [16,17] demonstrated that microvascular dysfunction occurs in the skeletal muscle microcirculation in septic rats following cecal ligation and puncture. In this rat model, the authors reported an increase in stopped-flow capillaries that was consistent with other reports [18], as well as an increase in the proportion of fast-flow to normal-flow capillaries and a decrease in capillary venular-end erythrocyte hemoglobin oxygen saturation levels, while capillary arteriolar-end erythrocyte hemoglobin oxygen saturation remained unchanged. Capillary oxygen extraction was found to increase threefold and be directly related to the degree of stopped flow. The authors concluded that the septic microcirculation could no longer regulate flow to regions of higher oxygen demand.

APC = activated protein C; FiO_2 = fraction of inspired oxygen; ICAM-1 = intercellular adhesion molecule-1; MAP = mean arterial pressure.

Similarly, animal models of sepsis have shown the existence of a greater number of adherent neutrophils in the coronary microcirculation [19,20], which is associated with increased heterogeneity in blood flow and impaired myocardial oxygen extraction [21]. Such observations in animal models are consistent with clinical observations of impaired oxygen extraction in human sepsis [22,23]. Microvascular dysfunction leading to impaired tissue oxygen extraction [24-26] has also been observed by several groups in the gut of animal sepsis models [3,24,27]. Increased heterogeneity of capillary blood flow, as measured by reduced capillary density, is a uniform link relating microvascular dysfunction to impaired oxygen extraction [24,26,28,29].

In a theoretical study, increased heterogeneity of capillary-bed blood flow was predicted to lead to regions of tissue hypoxia, and to a generally decreased ability of tissues to extract oxygen [24,26,28,29]. In this model, if some capillary beds have increased blood flow beyond their metabolic demand, while other capillary beds have reduced blood flow below their metabolic demand, then, when blood flow is limited, the onset of anaerobic metabolism in a substantial fraction of the involved tissue bed will occur early. The critical oxygen extraction ratio is, therefore, decreased in the setting of increased heterogeneity of microvascular blood flow. Humer and colleagues directly tested this theory in the porcine gut using an endotoxemic model of sepsis [24]. They showed that the increased heterogeneity of blood flow that occurs in a large-animal model of sepsis matched the predicted critical oxygen extraction ratio derived from the theoretical analysis. This suggests that microvascular dysfunction from sepsis leads to impaired extraction of tissue oxygen.

Microvascular dysfunction in humans

Clinically, microvascular dysfunction is seen in patients by using polarized light microscopy to examine sublingual microvessels. Using this technique, De Backer and colleagues have observed increased heterogeneity in microvascular blood flow in patients with septic shock [15]. Those patients who survived demonstrated improved microvascular function during the first few days of hospitalization, while no improvement in microvascular dysfunction was seen with time in non-survivors [13]. In essence, microvascular dysfunction occurs during sepsis and leads to impaired tissue oxygen extraction, causing heterogeneous tissue hypoxia that is spatially correlated with tissue inflammation. These observations are associated with an adverse clinical outcome.

Changes in endothelial cell signaling and function

Alterations in endothelial cell function underlie impaired microvascular blood flow (Fig. 1). Activation of the endothelium by circulating inflammatory cytokines leads to increased expression of endothelial adhesion molecules, including rapid expression of P-selectin and, later, an increase in expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 over several hours. Similarly, activated leukocytes increase their adherence to the endothelium by upregulating expression of cognate integrin receptors. In small vessels, leukocyte adhesion may contribute to disordered microvascular flow and potentially increase the inflammatory response within these local capillary beds by mediating the release of proinflammatory cytokines, reactive oxygen species, proteases and other inflammatory mediators.

Tyml and colleagues have identified disordered endothelial signaling function as an underlying mechanism leading to reduced microvascular regulation [30,31]. They describe how, in health, the endothelium produces signals upstream via tight junctions to precapillary arterioles so as to regulate blood flow to maintain homogeneity in normally functioning capillary beds. In models of sepsis, the authors showed that endothelial signaling is disrupted and leads to the impairment of precapillary arteriolar regulation.

Activation of this damaged endothelium produces an increased expression of adhesion molecules. This also leads to increased production of nitric oxide and impairment of the inflammation-related clotting system, including alterations in the expression of thrombomodulin and endothelial protein C receptor, and altered signaling via proteinase-activated receptor-1 and other pathways.

Tissue-based inflammation and resultant organ dysfunction

The capillary-endothelium-based inflammatory response, which is activated in sepsis, results in the generation of a systemic inflammatory response involving parenchymal cells in a variety of organ systems. In the heart, for example, cardiac myocytes release chemotactic cytokines. Then, fibrinogen extravasates into the extracellular space, which triggers cardiac myocytes to increase their expression of ICAM-1 so that the myocardium actively participates in directing an intraparenchymal inflammatory response. The parenchymal and capillary-endothelium-based inflammatory responses lead to impaired organ function.

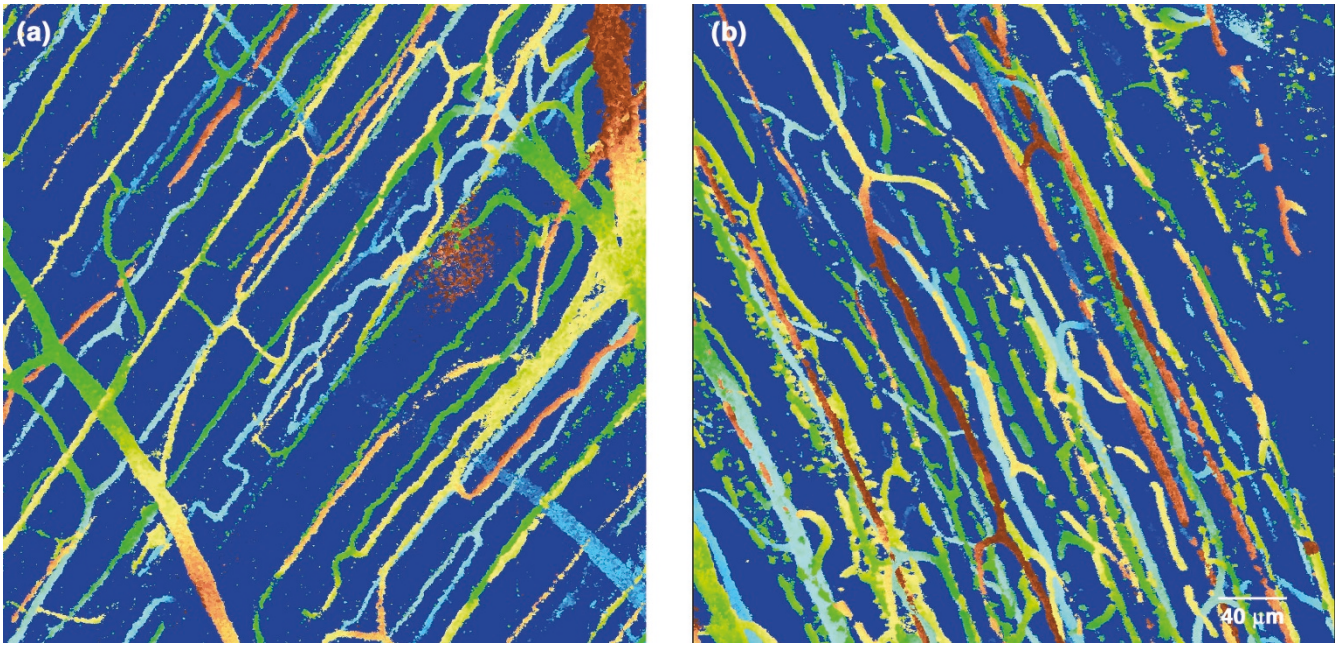
In the heart in sepsis, evidence of tissue hypoxia includes increased release of troponin. However, the appearance of regional abnormalities on echocardiographic examination in sepsis is not typical of occlusion of single major coronary arteries. Echocardiographic examination may disclose small regions of impaired function, resulting in an overall decrease in ejection fraction. This paradigm for impaired organ function in sepsis is also demonstrated by other organs, including the brain, the kidneys and the gut.

Therapeutic goals

Resuscitation of mean arterial pressure and cardiac output alone is not enough

As a therapeutic strategy, resuscitation of mean arterial pressure (MAP) and cardiac output alone is not enough to

Figure 1



Multiphoton images of CD1 mouse hind-limb extensor digitorum longus (EDL) skeletal muscle microcirculation. After blunt dissection of the EDL, mice were injected with fluorescent nanoparticles via the tail vein. Images were acquired by exciting tissue at 900 nm using a titanium sapphire laser and collecting the fluorescent signal using a Leica SP2 microscope (Lecia, Richmond Hill, Ontario, Canada). Images were pseudo colored for depth: brown represents the surface, green 75 µm depth and blue 150 µm depth. **(a)** The normal homogeneous appearance in control mice is disrupted **(b)** in mice 5 hours after endotoxin-treatment. Solid lines indicate perfused capillaries, whereas broken lines indicate stopped capillary flow.

resuscitate the microcirculation. Several pieces of evidence support this conclusion. An important study by LeDoux and colleagues [32] examined various hemodynamic parameters, as well as measures of tissue oxygenation, in patients treated with norepinephrine for septic shock. The investigators titrated the norepinephrine infusion to maintain a MAP of 65 mmHg, followed by 75 mmHg and then 85 mmHg. This combined α- and β-agonist led to increases in heart rate, MAP (by experimental design), and cardiac index ($P < 0.07$). The end result was a substantial increase in left ventricular stroke work index and a trend towards an increasing systemic vascular resistance index. Thus, from a purely hemodynamic perspective, norepinephrine appeared to be clinically beneficial. However, when the investigators looked for evidence of changes in organ function and adequate tissue oxygenation, the results were strikingly different: urine output decreased, capillary blood flow showed a trend to decreasing values, and capillary red blood cell velocity did not change. Furthermore, intramucosal partial pressure of carbon dioxide, measured using a gastric tonometer, and the gradient of pressure difference between arterial oxygen and inspired carbon dioxide both increased, indicating potentially impaired tissue oxygenation. Together, these results are evidence in human septic shock that resuscitation of MAP and cardiac output alone is inadequate.

Organ function improves when the microcirculation is also resuscitated

There is increasing evidence to show that organ function improves and mortality decreases when resuscitation boosts microcirculatory flow. Spronk and colleagues [14] demonstrated that the marked microvascular heterogeneity and dysfunction in patients with sepsis can be improved dramatically after a nitroglycerin infusion.

Microvascular function can, in animal models, be ameliorated by an adequate level of volume resuscitation. For example, volume resuscitation has been shown in studies by Anning and colleagues to decrease microvascular permeability in animal models of sepsis [33]. The same group demonstrated that, when microvascular function is improved by volume resuscitation in this way, tissue oxygenation improves [34]. In healthy animals, an increase in the fraction of inspired oxygen (FiO_2) results in an expected increase in tissue oxygenation, while in endotoxemic animals the same increase in FiO_2 has no effect on tissue oxygenation [34]. However, when endotoxemic animals were volume-resuscitated, an increase in FiO_2 resulted in an increase in tissue oxygenation.

Further animal studies of volume resuscitation have demonstrated that organ function is also improved [35]. A

series of studies has shown that cardiomyocyte contractility is decreased 5 hours after an endotoxin injection in rats [35]. However, when these rats are volume-resuscitated, cardiomyocyte function is improved. In these studies, albumin resuscitation appeared to be superior to saline resuscitation. Part of the improvement in myocardial function with albumin resuscitation appears to be related to a decrease in inducible nitric oxide synthase mRNA and protein expression in albumin-treated animals compared with other volume-resuscitated groups.

Strategies for resuscitating the microcirculation in septic patients

Combinational hemodynamic strategies

A number of strategies have emerged for resuscitating the microcirculation in septic patients. While vasopressor therapy alone is insufficient for resuscitation, it is now clear that the combination of adequate volume resuscitation, use of a vasopressor to maintain a reasonable MAP, and additional use of blood transfusion, inotropes or vasodilators to ensure adequate global oxygen delivery, is effective. This hemodynamic combinational strategy improves microcirculatory flow and organ function, and, ultimately, improves survival. Rivers and colleagues developed a protocol for this treatment in their "early goal-directed therapy" study [8]. They demonstrated that a combination of volume resuscitation to achieve a central venous pressure of 8–12 mmHg, addition of a vasopressor agent to maintain MAP >65 mmHg, measurement of central venous oxygen saturation, and use of transfused red blood cells and/or inotropic agents to increase central venous oxygen saturation to >70% brought about a substantially reduced mortality rate in patients with septic shock (30.5% for early goal-directed therapy versus 46.5% for standard therapy; $P = 0.009$).

Vasoactive agents

In addition, alternative vasoactive agents should be considered. For example, in an early randomized controlled trial of vasopressin, Patel and colleagues found that equally hypertensive doses of norepinephrine and vasopressin maintained cardiac output to the same extent, yet urine output and creatinine clearance were increased in the vasopressin-treated group [36]. Previous studies in rats have demonstrated that vasopressin differs from norepinephrine in its effects on the microcirculation [37]. The afferent glomerular arterioles constrict more readily when exposed to norepinephrine than vasopressin, whereas the efferent glomerular arterioles constrict more readily when exposed to vasopressin than norepinephrine [37]. An increased glomerular perfusion pressure and increased urine output is the result. This demonstrates how different vasopressor agents affect the microcirculation to a varying degree. Further studies are required to determine which vasopressor strategies should best be used to treat septic shock.

Modulation of the inflammatory response

Modulation of inflammation is an important strategy when considering microvascular resuscitation. As discussed earlier, the endothelium is actively involved in the systemic inflammatory response of sepsis and plays a key role in normal microvascular regulation, in interactions with potentially detrimental circulating leukocytes, and as the origin of a tissue-based inflammatory response that ultimately leads to organ dysfunction. Corticosteroid therapy represents a non-specific approach to modulation of the systemic inflammatory response, and has important anti-inflammatory effects on the endothelium. Annane and colleagues found that more than half of all patients with septic shock had an inadequate adrenal response to adrenocorticotrophic hormone [38]. These patients responded well to low-dose hydrocortisone (50 mg administered intravenously every 6 hours), and their mortality decreased from 63% to 51%. However, indiscriminant steroid use is not benign, and previous studies have shown that high-dose corticosteroid therapy in septic shock increases mortality [38]. Annane and colleagues demonstrated that adrenocorticotrophic hormone responders had reduced survival when treated with steroids [38].

Anticytokine therapy is an alternative approach to modulation of the systemic inflammatory response, with subsequent endothelial effects. While a large number of anticytokine therapies have failed or have shown only marginal success in phase III randomized, controlled trials, several recent meta-analyses indicate that this strategy may be successful [39]. When the most severely ill patients are considered, these anticytokine therapies have been uniformly successful in decreasing mortality and measures of organ dysfunction [39]. Patient selection is critically important in ensuring that these promising new therapies are properly implemented. Those patients at high risk of an adverse outcome constitute the target population.

Targeting the endothelium

The microcirculation in septic patients can also be resuscitated by specifically targeting the endothelium using a strategy that reduces inflammation and coagulation while increasing fibrinolysis. This has led to the first successful phase III randomized, controlled trial in a severe sepsis patient population using exogenous activated protein C (APC) to treat the uncontrolled cascade of inflammation, coagulation, and impaired fibrinolysis that occurs [40]. Exogenous APC has specificity for key steps in the inflammatory response and coagulation cascade that occurs at or near the endothelial surface [41,42]. Through specific anti-inflammatory effects, or as a result of its combined actions at the endothelial cell luminal surface, exogenous APC decreases organ dysfunction and improves survival in patients with severe sepsis [6]. This provides further evidence that the endothelium and, by inference, the microvasculature play a key role in the pathogenesis and outcome from sepsis.

Conclusions

There are many data to show that microvascular dysfunction contributes to higher mortality rates in sepsis. Impaired microvascular blood flow in sepsis is caused by an alteration in endothelial function and signaling, leading to some capillary beds having reduced or even stopped blood flow, whilst others have increased blood flow beyond their metabolic demand. This heterogeneity in microvascular perfusion leads to some areas of tissue receiving less oxygen than they require, and results in regional hypoxia. The septic alterations in endothelial signaling also result in an inflammatory response which, in combination with hypoxia, can lead to impaired organ function that has a major correlation with mortality in sepsis.

A major therapeutic goal of treating sepsis is therefore to restore normal microvascular function. Some animal studies have shown that volume resuscitation can be effective in restoring microvascular function; however, clinical studies suggest that, in humans, this strategy is not enough. A combinational strategy involving volume resuscitation, vasopressors, and use of transfused red blood cells and/or inotropic agents has been shown to improve survival. In addition, mediation of inflammation is important, and studies have shown that corticosteroids and anticytokine therapy might be useful. Finally, therapies that specifically target the endothelium might be able to reduce inflammation and coagulation whilst increasing fibrinolysis. Studies of exogenous APC have shown that this agent is successful in reducing mortality in severe sepsis patients.

Competing interests

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