Review

The microcirculation as a functional system

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Abstract

This review examines experimental evidence that the microvascular dysfunction that occurs early in sepsis is the critical first stage in tissue hypoxia and organ failure. A functional microvasculature maintains tissue oxygenation despite limitations on oxygen delivery from blood to tissue imposed by diffusion; the density of perfused (functional) capillaries is high enough to ensure appropriate diffusion distances, and arterioles regulate the distribution of oxygen within the organ precisely to where it is needed. Key components of this regulatory system are the endothelium, which communicates and integrates signals along the microvascular network, and the erythrocytes, which directly monitor and regulate oxygen delivery. During hypovolemic shock, a functional microvasculature responds to diminish the impact of a decrease in oxygen supply on tissue perfusion. However, within hours of the onset of sepsis, a dysfunctional microcirculation is, due to a loss of functional capillary density and impaired regulation of oxygen delivery, unable to maintain capillary oxygen saturation levels and prevent the rapid onset of tissue hypoxia despite adequate oxygen supply to the organ. The mechanism(s) responsible for this dysfunctional microvasculature must be understood in order to develop appropriate management strategies for sepsis.

Introduction

One of the primary functions of the microcirculation is to ensure adequate oxygen delivery to meet the oxygen demands of every cell within an organ. In order to achieve this, the healthy microvasculature will respond to changes in metabolic demand or blood flow to the organ. However, if the microvasculature is dysfunctional, as it is in sepsis, then tissue hypoxia can occur despite supranormal oxygen delivery values. In order to understand how sepsis can result in tissue hypoxia in organs remote to the initial site of injury, we first need to understand oxygen transport and the regulation of oxygen delivery under normal physiological conditions.

Normal physiology

Diffusion limitation for oxygen

More than 80 years ago, Krogh [1] published the first oxygen transport model that described diffusion of oxygen from a single capillary cross-section into the surrounding cylinder of tissue. This model highlighted the impact of diffusion limitation on tissue oxygenation and hence explained why capillary density was greater in tissues with higher oxygen consumption rates. The model also demonstrated that it is not sufficient to simply supply an adequate amount of oxygen to the organ as a whole, but that oxygen must be distributed within the organ precisely to where it is needed.

Integration of arteriolar regulation

Arterioles, which control the vascular resistance of an organ and hence its total blood flow, are also responsible for regulating the distribution of oxygen within the organ itself. To achieve this degree of control, the response of the microvasculature to changing conditions (e.g. increased oxygen demand, reduced oxygen delivery) must be highly integrated across the entire microvascular bed [2-4]. The endothelial cells play a critical role in conducting and integrating local stimulatory signals via cell-to-cell communication along the microvascular endothelium [5-7] or by responding to changes in blood flow as signal transducers of local shear stress [8]. For example, if there is a dilatory stimulus originating in one region of the capillary bed, the vascular endothelium will conduct this stimulus to the arterioles supplying these capillaries, causing them to dilate, thus increasing blood flow. Endothelium lining larger arterioles and resistance arteries further upstream will respond to the increase in shear stress by dilating to the point that local shear stress is restored back to baseline, and thus further reducing vascular resistance.

ATP = adenosine triphosphate; ENHANCE = Extended Evaluation of Recombinant Human Activated Protein C; iNOS = inducible form of nitric oxide synthase; LPS = lipopolysaccharide; NO = nitric oxide; PROWESS = Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis; RBC = red blood cell; SNO = S-nitrosothiol.

Without this integrated response, a local dilatory stimulus could "steal" flow from other regions of the tissue.

Precapillary fall in oxygen saturation

Thirty-five years ago, Duling and Berne [9] reported that oxygen levels diminished along the arteriolar tree and that up to two-thirds of the oxygen delivered to a tissue has already been extracted by the time blood reaches the capillary bed. Using a variety of techniques in different organs and species, numerous researchers have documented these experimental observations [10,11]. Although we do not fully understand why there is such a large precapillary decrease in oxygen, Ellsworth and Pittman [12] provided experimental evidence to show that some of the oxygen leaving the arterioles can reoxygenate red blood cells (RBCs) flowing through nearby capillaries by diffusion. If oxygen can be transported from arterioles to capillaries, it is also likely that oxygen exchange occurs between capillaries with different oxygen levels [10], and between arterioles and venules [13]. In addition, quantitative studies of microvascular blood flow have demonstrated considerable spatial heterogeneity of capillary perfusion [14,15]. The unique rheological properties of RBC flow through branching networks of small vessels (Fahreaus effect and plasma skimming at bifurcations [16]) results in wide distributions of capillary hematocrits and RBC flow rates. The heterogeneity of microvascular hematocrit, the precapillary drop in oxygen saturation, and the diffusional exchange of oxygen among microvessels mean that blood flow by itself is not a good indicator of adequate oxygen delivery to tissue. This has important implications for the regulation of the oxygen supply, particularly during disease states and the investigation of microvascular oxygen delivery *in vivo*.

The role of RBCs in local regulation of oxygen delivery

The automatic feedback system responsible for regulating local oxygen delivery must be able to monitor and regulate oxygen delivery throughout the microvascular bed. Bergfeld and Forrester [17] were the first to demonstrate that RBCs exposed to hypoxic conditions released adenosine triphosphate (ATP). Since ATP is a potent vasodilator, they proposed that RBCs flowing through a hypoxic region could stimulate local vasodilation and an increase in blood flow. Ellsworth and colleagues [18,19] demonstrated that ATP injected into arterioles results in local vasodilation that is also conducted along the arteriole, thus demonstrating the presence of purinergic receptors (P_{2y1} and P_{2y2}) on the endothelium of these vessels. ATP binding to P_{2y1} and P_{2y2} on vascular endothelium causes vasodilation of vascular smooth muscle by inducing the endothelium to produce nitric oxide (NO) [20], prostaglandin [21], or endothelium-derived hyperpolarizing factor [22,23]. Collins and colleagues [24] demonstrated that ATP injected into postcapillary venules results in vasodilation of the feeding arteriole. Dietrich and colleagues [25] showed that isolated cerebral arterioles dilate in response to a fall in oxygen in their environment only if the arterioles are perfused with RBCs, and not if they are perfused with a physiological solution without RBCs. They also observed that this vasodilation was caused by the efflux of ATP from the RBCs [25], and demonstrated that the oxygen-dependent release of ATP occurred rapidly enough to be physiologically relevant. Jagger and colleagues [26] have shown that ATP efflux is linearly related to hemoglobin oxygen saturation and that the regulation of glycolysis by deoxyhemoglobin in RBCs is the first step in the signaling pathway for ATP release. Also, ATP injected into larger venules results in vasodilation of the paired arteriole [27-29]. Saltin and colleagues, studying exercising human volunteers, have reported that ATP released from RBCs in response to a fall in hemoglobin oxygen saturation was responsible for regulating oxygen delivery to skeletal muscle [30,31].

In 1996, Stamler and his colleagues [32] also proposed that RBCs are responsible for regulating oxygen delivery through the transport of NO, produced in the lungs, to the periphery in the form of the bioactive compound S-nitrosothiol (SNO). SNO, reported to be a potent vasodilator, is carried by hemoglobin and released as the hemoglobin oxygen saturation falls in response to local oxygen demand. Although Stamler's group have published numerous papers supporting their theory [33,34], a number of groups have questioned the physiological role of SNO *in vivo* [35,36] as well as the accuracy of measurements of SNO from biological samples [37]. In 2003, Cosby and colleagues [38] reported that deoxyhemoglobin acts as a nitrite reductase, converting nitrite to NO, and hence making it possible for RBCs to vasodilate arterioles in response to hypoxia.

The potential for hemoglobin to play a key role in regulating vascular tone and hence oxygen delivery has generated considerable excitement [39], and has elevated the RBC from a simple carrier of oxygen to a cell ideally suited to monitor and regulate oxygen delivery across the entire microvascular bed [40].

Sepsis and microvascular dysfunction

What is the cause of organ failure in sepsis? A review article from 2000 suggests that clinical and experimental evidence "clearly indicate that microcirculatory dysfunction lies at the centre of sepsis pathogenesis" [41].

Loss of capillaries in remote organs

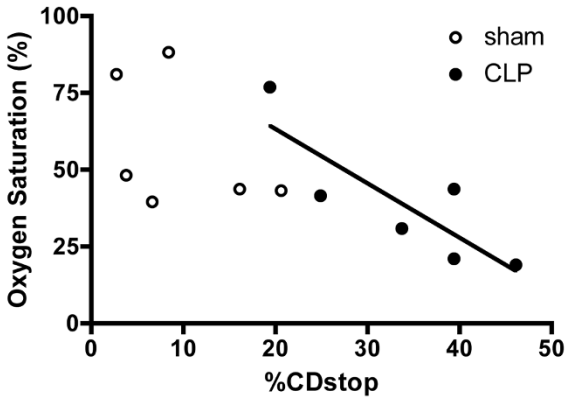
In 1994, Lam and colleagues [42] reported that a 24-hour peritonitis model of sepsis (cecal ligation and puncture) in rats caused a decrease in the number of perfused capillaries (i.e. decrease in functional capillary density) in skeletal muscle, with increased heterogeneity of blood flow. The loss of perfused capillaries in experimental models of sepsis has been reported in the microvasculature of intestinal villi [43,44], the diaphragm [45], and the liver [46].

Maldistribution of oxygen delivery

Using intravital video microscopy, we have studied the impact of the loss of capillary density on capillary oxygen saturation

in a fluid resuscitated, normotensive, peritonitis model of sepsis similar to that used by Lam and colleagues [42]. Using a dual-wavelength system for spectrophotometric analysis of RBC oxygen saturation, video images of microvascular blood flow were analyzed for perfused capillary density, RBC hemodynamics, and the oxygen saturation levels at the entrance and exit of the capillary bed [47]. This study confirmed the presence of stopped-, normal-, and high-flow capillaries in the same field of view. We demonstrated that the loss of capillaries (from 20% to 50% stopped flow) leads to a significant fall in oxygen saturation in normally perfused capillaries (from 60% to 20% saturation) and an increase in capillary oxygen extraction [47], as shown in Fig. 1. There was no evidence that the local oxygen regulatory system was effective in redistributing oxygen supply to offset the fall in capillary oxygen saturation levels, a result that is in accordance with the reported impaired hyperemic response to exercise observed by Lam and colleagues in the same sepsis model [42].

Figure 1



Oxygen saturation of red blood cells at the venous end of normally perfused capillaries versus the percentage of capillaries with stopped-flow (%CDstop) in extensor digitorum longus muscle in rat. No relationships existed in the sham animals between these parameters. In animals that underwent a 24-hour peritonitis model of sepsis (cecal ligation and perforation [CLP]), there was a decrease in oxygen saturation with increasing %CDstop (linear regression: $y = 98.8 - 1.8x$; $r^2 = 0.64$; $P < 0.05$). Reproduced with permission [47].

Hypovolemic shock versus septic shock

The situation is very different if the microvasculature is still functional and able to regulate oxygen distribution within the capillary bed. Nakajima and colleagues [44] compared microvascular perfusion in intestinal villi in mouse models of septic shock and hypovolemic shock (hemorrhage). They demonstrated that, at the same level of hypotension, hemodynamic and mucosal perfusion disorders were considerably more pronounced in endotoxin-induced hypotension than in hemorrhagic hypotension. RBC velocity was maintained in hemorrhagic shock but not during septic shock. During hypovolemic shock the microvasculature was still able to regulate microvascular perfusion, but during sepsis the regulatory response was impaired.

Experiment-based mathematical model of oxygen transport in sepsis

Our simple interpretation of the increase in oxygen extraction following a loss of perfused capillaries in sepsis was that each perfused capillary would need to support a larger volume of tissue to compensate for the loss of oxygen supply from stopped-flow capillaries [47]. However, this interpretation did not take into account the possibility of an increase in oxygen consumption rate or the potential contribution of oxygen from fast-flow capillaries. To address this limitation, Goldman and colleagues [48] developed a mathematical model of capillary oxygen delivery in a three-dimensional volume of tissue that was based on our experimental data on capillary hemodynamics and oxygen saturation in sepsis. Tissue oxygen consumption rates were adjusted in the model to yield oxygen extraction values that were consistent with our experimental measurements of capillary oxygen extraction. The model predicted that oxygen consumption increases from between two- to fourfold depending upon the severity of sepsis, and that the loss of perfused capillaries leads to significant tissue hypoxia but not to anoxia. Despite the loss of capillaries and increased oxygen consumption, the model predicted that the tissue is protected from zero oxygen levels by the high-flow capillaries that supply a substantial fraction of the total oxygen delivered to the tissue. However, these high-flow capillaries do have higher venular end-oxygen saturations than normal-flow capillaries, and hence "shunt" oxygen through the capillary bed, thus elevating venular oxygen saturation levels. If the excess oxygen carried by these capillaries is uniformly distributed to all perfused capillaries, then the fall in tissue oxygen levels would be less.

Implications from experimental and mathematical models of sepsis

Based on our experiments and mathematical model, we propose that loss of perfused capillaries and impaired regulation of oxygen delivery within the microcirculation leads to a maldistribution of microvascular blood flow and tissue hypoxia early in sepsis, and that this is the first step in the progression to organ failure [49]. The tissue is still capable of extracting oxygen, but oxygen is not being delivered to where it is needed. Early in sepsis, the inability of the microvasculature to compensate for a loss of functional capillary density is the critical factor that leads to tissue hypoxia and thus organ dysfunction.

Clinical relevance

Are these results from our experimental models of sepsis clinically relevant? Using orthogonal polarization spectral imaging, De Backer and colleagues demonstrated that the density of perfused capillaries in sublingual tissue was reduced in septic patients [50], similar to what we have

observed in our animal models. Recently, this group has reported that survivors of septic shock show an improvement in perfused capillary density, but those who die have a persistent loss of perfused capillaries [51]. The loss of perfused capillaries in organs remote to the initial site of inflammation occurs in septic patients and may be an important indicator of outcomes. The key questions from an oxygen transport perspective are why does capillary blood flow stop in sepsis and why has the local oxygen regulatory system not responded to the fall in capillary oxygen saturation by distributing blood flow and oxygen to where it is needed?

Mechanisms underlying the maldistribution of oxygen delivery in sepsis

Occlusion of capillaries

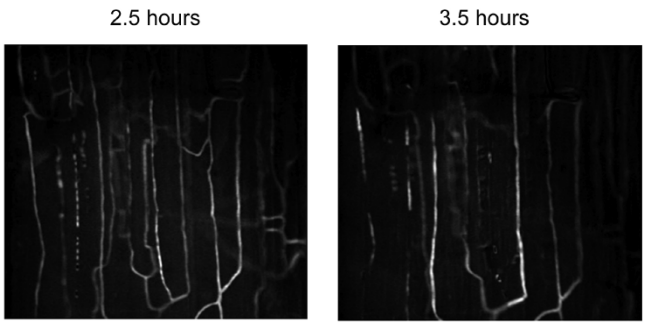
There are several proposed mechanisms for the occlusion of capillaries early in sepsis: stiff leukocytes, stiff RBCs, endothelial cell swelling, and platelet/fibrin clots [49].

Piper and colleagues [52] investigated the time course (from 6–48 hours) of leukocyte rolling, adhesion, and extravasation in postcapillary venules in skeletal muscle using the same peritonitis model of sepsis as that of Lam and colleagues [42]. Although Piper and colleagues observed an increase in rolling at 24 hours, they found that leukocyte adhesion in venules was reduced due to a fall in circulating white blood cell count. However, Goddard and colleagues presented evidence from endotoxemia models of sepsis that leukocytes have a prolonged capillary transit time and are retained in the coronary capillaries of pigs [53] and rabbits [54], making the leukocyte a good candidate for occluding capillaries. Although the results of Piper and colleagues might at first seem to contradict that of Goddard and colleagues, both studies support the concept that the loss of capillaries is not due to occlusion of venules by an accumulation of leukocytes but due to the direct occlusion of capillaries.

We developed a 6-hour peritonitis model of sepsis in the rat to follow the progression of remote inflammatory injury in skeletal muscle (Fig. 2). Using this model, Bateman and colleagues [55] observed that the time course for loss of RBC deformability, excess NO production, and increased numbers of stopped-flow capillaries were correlated. Treatment of the septic rats with aminoguanidine (an inhibitor of the inducible form of NO synthase [iNOS]) to maintain plasma nitrite/nitrate levels at baseline prevented the loss of RBC deformability and the loss of perfused capillaries [55]. Our report of a subpopulation of RBCs with very low deformability at 37°C [55,56] very early in sepsis was recently confirmed [57]. These results support the role of stiff RBCs in capillary plugging [49].

There is convincing evidence that disseminated intravascular coagulation plays a central role in organ failure in sepsis [58]. Treatment of severely septic patients with activated protein C, which targets both the coagulation and inflammation pathways in sepsis, has been shown to be effective in reducing mortality [59,60]. Although the success of the Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) and Extended Evaluation of Recombinant Human Activated Protein C (ENHANCE) trials supports the possibility of platelet/fibrin clots impairing microvascular perfusion, experimental studies are needed to further elucidate the mechanisms of action of activated protein C on the microcirculation during the early stages of sepsis.

Figure 2



Functional images of the same capillary bed in the extensor digitorum longus muscle of the rat at 2.5 and 3.5 hours after induction of a peritonitis model of sepsis (cecal ligation and perforation [CLP]). The functional images were generated from captured video sequences (30 seconds) and show those capillaries through which red blood cells were flowing. At 2.5 hours after CLP, most capillaries in the field of view are perfused. One hour later, individual capillary segments from within the capillary network no longer have red blood cell flow, indicating the rapid progression of the remote injury to the microvasculature of this muscle. The procedure used for generating functional (variance) images was described by Japee *et al.* [70].

It is likely that a combination of these mechanisms contributes to the loss of functional capillary density in sepsis. Since the loss of capillaries in remote organs begins to occur several hours after the initial injury, and hence several hours after leukocyte activation, we speculate that activation and/or injury of the microvascular endothelium in remote organs is the critical first step leading to capillary loss.

Impaired local regulation of oxygen delivery

In addition to an impaired arteriolar response to vasoactive stimuli in animal models of sepsis [61-63], Tyml and colleagues have shown that there is impaired communication of signals between endothelial cells in culture exposed to lipopolysaccharide (LPS) [64,65] and along the vascular endothelium *in vivo* in peritonitis [62] and LPS models of sepsis [64,66]. The mechanism responsible for impaired arteriolar responsiveness to stimuli appears to be excess NO production in endothelial cells via iNOS [67]. Impaired communication along the vascular endothelium is reported to be due to an LPS-induced increase in intercellular resistance [64] that may be mediated by tyrosine phosphorylation of

connexin 43, a gap-junction molecule [68,69]. The inability of the arteriolar tree to properly integrate its response to the tissue's needs may be a significant factor in the maldistribution of oxygen delivery to tissue in sepsis. We can also speculate that erythrocyte injury in sepsis, as indicated by a loss of RBC deformability, may mean that the ability of RBCs to regulate oxygen delivery through ATP release is also impaired.

Conclusion

In metabolically active tissue, diffusion limitation places strict constraints on how far cells can be from an oxygen source. This determines not only functional capillary density but also the characteristics of the microvascular control systems. Vascular endothelium and RBCs play a significant role in coordinating the response of the arteriolar tree to changes in oxygen demand or oxygen delivery to the organ. As long as the regulatory system is functional and capillary density is sufficient, the microvasculature will deliver all available oxygen to where it is needed within an organ. In hemorrhagic shock, a "functional" microvasculature reduces the impact of a decrease in oxygen supply on tissue hypoxia by efficiently distributing oxygen to where it is needed. During the early stages of sepsis, however, the loss of capillary density and the impaired ability to regulate local oxygen delivery results in the rapid onset of tissue hypoxia despite more than adequate oxygen supply to the organ. Clearly we need to understand the mechanism(s) responsible for this dysfunctional microvasculature in order to develop appropriate management strategies for sepsis.

Competing interests

CGE received reimbursement for travel expenses and an honorarium for speaking at the Global Medical Conference in Brussels 2005 and for preparing this manuscript. CGE's sepsis research is supported by an operating grant from the Canadian Institutes for Health Research (MOP-49416), and his research on the erythrocyte role in local regulation of oxygen delivery by an operating grant from Heart and Stroke Foundation of Ontario.

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