Commentary

Nitric oxide, leukocytes and microvascular permeability: causality or bystanders?

Balázs Hauser[1,2], Martin Matejovic[3] and Peter Radermacher[1]

[1]Sektion Anästhesiologische Pathophysiologie und Verfahrensentwicklung, Universitätsklinikum, Parkstrasse 11, 89073 Ulm, Germany
[2]Aneszteziológiai és Intenzív Terápiás Klinika, Semmelweis Egyetem, H-1125 Kietvolgyi, Budapest, Hungary
[3]1. Interni klinika, Karlova univerzita Praha, Lekarska fakulta a Fakultni nemocnice, Allej Svobody 80, 30460 Plzen, Czech Republic

Corresponding author: Peter Radermacher, peter.radermacher@uni-ulm.de

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Abstract

Increased microvascular permeability resulting in tissue edema is a hallmark of sepsis-related microcirculatory failure, and leukocyte–endothelium interaction is thought to assume major importance in this context. However, the role of nitric oxide (NO) in the interplay of inflammation, leukocyte–endothelium interaction and increased microcirculatory permeability is still a matter of debate. Hollenberg *et al.* now report, in the previous issue of *Critical Care*, that neither genetic deletion nor pharmacologic blockade of the inducible isoform of the NO synthase (iNOS) affected the sepsis-related aggravation of leukocyte rolling and adhesion, whereas iNOS inhibition attenuated microvascular permeability. The authors conclude that excess NO resulting from iNOS activation is important in modulating vascular permeability during sepsis, but that this effect is independent of its action on leukocytes.

Increased microvascular permeability resulting in tissue edema is a hallmark of sepsis-related microcirculatory failure, and in this context leukocytes are thought to assume major importance. However, the role of nitric oxide (NO) in the interplay of inflammation, leukocyte–endothelium interaction and increased microcirculatory permeability is still a matter of debate. It is well established that NO has a pivotal role in the regulation of vasomotor tone as well as in host defense and immune function, and abundant literature is available on both its protective and its detrimental properties, which depend on the source of its release (for example, isoenzyme activation), the timing and the amount of its production, and the redox status of the surrounding milieu. In the previous issue of *Critical Care*, Hollenberg *et al.* [1] added another piece to this complex puzzle. Using a well-established, clinically relevant murine model of resuscitated, hyperdynamic sepsis resulting from cecal ligation and puncture (CLP) [2], the authors studied the effects of both genetic deletion and pharmacologic blockade of the inducible isoform of the NO synthase (iNOS) on leukocyte adhesion and rolling as well as on microvascular leakage. In this model, the authors had previously shown that iNOS$^{-/-}$ mice presented with improved microvascular catecholamine responsiveness and, ultimately, enhanced survival [2]. As expected, in the present study CLP itself aggravated leukocyte rolling and adhesion. Interestingly, deletion of iNOS did not affect this response, whereas it attenuated microvascular permeability. In sham-operated control mice, iNOS-derived NO inhibited the interaction between leukocytes and endothelial cells (rolling and adhesion), but not microvascular permeability. The authors concluded that excess NO resulting from iNOS activation is important in modulating vascular permeability during sepsis, but that this effect is independent of its action on leukocytes.

How do these findings compare with the available literature on the role of NO in leukocyte–endothelium interaction and microvascular permeability?

More than a decade ago, Kubes *et al.* showed that non-selective NO synthase inhibition increased leukocyte adherence [3]. This effect was closely related to oxidative stress resulting from an enhanced production of superoxide radicals [4], thus demonstrating the importance of NO as an oxygen radical scavenger. In rats with CLP, non-selective NO synthase inhibition also increased leukocyte migration [5]. Activation of iNOS seemed to be responsible for the protective properties of NO, because iNOS$^{-/-}$ mice challenged with lipopolysaccharide presented with a comparably increased accumulation of pulmonary leukocytes [6]. Furthermore, iNOS$^{-/-}$ caused enhanced pulmonary inflammation after instillation of lipopolysaccharide into the lung [7], whereas wild-type controls and mice lacking endothelial NO synthase (eNOS$^{-/-}$) presented with similar less pronounced

CLP = cecal ligation and puncture; eNOS = endothelial nitric oxide synthase; iNOS = inducible nitric oxide synthase; NO = nitric oxide.

inflammatory responses. Finally, iNOS$^{-/-}$ mice subjected to lethal CLP showed even more pronounced leukocyte rolling and adhesion than wild-type controls treated with the non-selective NO synthase blocker aminoguanidine [8]. Because NO affected only minimally the most important adhesion molecules (P-selectin, E-selectin and vascular cell adhesion molecule-1) regulating leukocyte response, Hickey *et al.* concluded that iNOS-related changes affecting leukocyte behavior in the microcirculation are due to an altered leukocyte function rather than an altered endothelial function [9].

The picture is far less straightforward with regard to microvascular permeability. Clearly, Kubes and Granger [10] elegantly demonstrated that the non-selective NO blockade markedly increased fluid leakage into the extravascular space. This effect was due not only to increased microvascular hydrostatic pressure but also to increased microvascular permeability [10]. Nevertheless, Paul Kubes also emphasized the 'continuing dilemma of NO and microvascular permeability' [11] due to the compelling evidence that endogenous NO may either decrease or increase fluid leakage [12]. In fact, even inhaled NO was reported to increase epithelial permeability and alveolar fluid leakage in rats with pneumonia [13], a rather intriguing observation because Benzing *et al.* [14] had reported decreased transvascular albumin flux in patients with acute lung injury, which was due at least in part to a fall in the pulmonary effective capillary pressure; that is, the microvascular hydrostatic pressure. Differentiating between the constitutive endothelial, and inducible NO synthase isoforms, namely eNOS and iNOS, adds to the complexity: the present data by Hollenberg *et al.* clearly indicate a major role of iNOS, whereas other authors investigating both eNOS$^{-/-}$ and iNOS$^{-/-}$ animals found that iNOS was associated with protective rather than deleterious properties; in fact, in the present study, iNOS$^{-/-}$ mice challenged with CLP presented with less microvascular leakage, whereas eNOS$^{-/-}$ mice were protected against tissue edema after intrapulmonary instillation of lipopolysaccharide [7], zymosan injection into the paw [15], and injection of platelet-activating factor into the mesentery [16].

Consequently, what do we learn for clinical practice? Or, in other words, shall we say 'No to iNOS' so as to attenuate sepsis-induced tissue edema resulting from microvascular failure? Data from clinically relevant, resuscitated models of both genetic and pharmacologic iNOS inhibition [1,2] clearly favor this approach, and similar conclusions can be drawn from existing reports on large animals [17]. Nevertheless, it must be emphasized that 'a mouse is not a man': in rodents, endogenous NO production is higher by one or two orders of magnitude than in human beings [18]. In addition, it must be noted that up to now clinical data have not supported a direct relation between NO release and the capillary filtration coefficient, a noninvasive index of microvascular permeability [19]. Consequently, a definite answer is still lacking.

Competing interests

The authors declare that they have no competing interests.

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