Nitric oxide in shock

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Refractory hypotension with end-organ hypoperfusion and failure is an ominous feature of shock. Distributive shock is caused by severe infections (septic shock) or severe systemic allergic reactions (anaphylactic shock). In 1986, it was concluded that nitric oxide (NO) is the endothelium-derived relaxing factor that had been discovered 6 years earlier. Since then, NO has been shown to be important for the physiological and pathological control of vascular tone. Nevertheless, although inhibition of NO synthesis restores blood pressure, NO synthase (NOS) inhibition cannot improve outcome, on the contrary. This implies that NO acts as a double-edged sword during septic shock. Consequently, the focus has shifted towards selective inducible NOS (iNOS) inhibitors. The contribution of NO to anaphylactic shock seems to be more straightforward, as NOS inhibition abrogates shock in conscious mice. Surprisingly, however, this shock-inducing NO is not produced by the inducible iNOS, but by the so-called constitutive enzyme endothelial NOS. This review summarizes the contribution of NO to septic and anaphylactic shock. Although NOS inhibition may be promising for the treatment of anaphylactic shock, the failure of a phase III trial indicates that other approaches are required for the successful treatment of septic shock. Amongst these, high hopes are set for selective iNOS inhibitors. But it might also be necessary to shift gears and focus on downstream cardiovascular targets of NO or on other vasodilating phenomena.

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SHOCK: CAUSES AND EPIDEMIOLOGY

Shock may be defined as the failure of the circulation to provide sufficient blood and oxygen to peripheral organs. Key symptoms of shock are severe hypotension and vasoplegia, ultimately resulting in the dysfunction of one or more vital organs, such as kidney, liver, gut, lung, and brain. Life-threatening shock may be caused by acute myocardial infarction (cardiogenic shock), severe fluid or blood loss (hypovolemic or hemorrhagic shock), severe infection (septic shock), or severe allergic reaction (anaphylactic shock). The most common type of shock is hemorrhagic shock; in children, elderly, and immunocompromized people, septic shock is the most common. In the first week after diagnosis, refractory hypotension is the leading cause of death; later on, death is generally caused by multiple organ failure as a result of prolonged hypotension and cytotoxicity. The history of clinical trials in septic patients extends back to 1963, when high-dose hydrocortisone was used.[1] But despite almost half a century of clinical trials, and more than two decades of extensive research, only two experimental approaches have survived the numerous clinical trials and have reached the septic patient: low-dose corticosteroids and recombinant human activated protein C.[1,2] Still, their beneficial effect on survival seems to depend on the severity of the illness and they may be rather harmful in patients with a lower risk of death.[2] In addition, recent trials failed to show any significant benefit of recombinant human activated protein C and indicated an increased risk of bleeding, making it unclear whether its alleged beneficial effects in fact outweigh its risks.[3] Thus, severe sepsis and septic shock are still associated with an unacceptably high mortality rate of 50–70%. Short-term mortality from septic shock has decreased in recent years. In one study, for example, mortality fell from 62% in the early 1990s to 56% in 2000.[4] Nevertheless, overall mortality is increasing, as the incidence of sepsis is growing by 9% each year.[4,5] Consequently, these days more people die annually from septic shock than from myocardial infarction, lung or breast cancer, stroke, or trauma.[6] Anaphylaxis can occur in response to any allergen, most commonly insect stings, food, and drugs such as antibiotics, contrast materials, and anesthetics. In general, about 1% of people with an allergic history are prone to anaphylaxis, but some authors consider up to 15% of the US population 'at risk'.[7] Overall, the frequency of anaphylaxis is increasing because of the

soaring incidence of allergies and the increased number of potential allergens to which people are exposed.

NITRIC OXIDE: HISTORY AND BACKGROUND

In 1980, Furchgott and Zawadzki[8] reported that endothelial cells release a labile factor that causes blood vessel relaxation. In 1986, it was suggested, and subsequently confirmed, that this endothelial-derived relaxing factor is the short-lived, gaseous, highly reactive radical nitric oxide (NO).[9–13]

NO is produced enzymatically by three different NO synthases (NOS). Neuronal NOS (nNOS) (NOS1) and endothelial NOS (eNOS) (NOS3) are constitutive enzymes important for homeostatic processes, such as neurotransmission and vascular tone, respectively. They produce small amounts of NO in response to increases in intracellular calcium. More recently, the constitutive nature of eNOS has achieved new dimensions, as it became clear that the enzyme's activity may be regulated, both transcriptionally and post-transcriptionally, with acylation, phosphorylation, subcellular localization, and protein interactions determining its activity.[14] The third enzyme, inducible NOS (iNOS) (NOS2), is normally not expressed, but is synthesized *de novo* in response to inflammation. It is calcium-independent and produces large amounts of NO over prolonged periods of time.[15] NOS enzymes make NO from L-arginine, and thus competitive L-arginine analogues may prevent them from producing NO. These analogues include N^G-monomethyl-L-arginine (L-NMMA), N^G-nitro-L-arginine (L-NNA), and N^G-nitro-L-arginine methyl ester (L-NAME). As early as 1989, some of these compounds were already successfully used to demonstrate the important physiological role of NO in normal blood pressure homeostasis.[16,17]

NO IN SEPTIC SHOCK: CRITICAL MEDIATOR OF HYPOTENSION

Shortly after the discovery that NO is an important endogenous regulator of vascular tone, its fundamental contribution to inflammatory and septic shock became obvious as well. The NO metabolites nitrite and nitrate (collectively labeled NO_x^-), indicators of NO production, rise progressively in various animal shock models.[18] In small rodents, plasma concentrations of hundreds to even thousands micromolar may be detected. In larger mammals and humans, however, overproduction does not occur to the same extent and levels rarely increase above 100 μM, or more than 50% above background, despite major circulatory failure.[18] Nevertheless, the critical role of NO in shock has been clearly established, as NOS inhibitors prevent, revert, or at least minimize hypotension in shock induced by lipopolysaccharide (LPS), tumor necrosis factor (TNF), interleukin-1, interleukin-2, or hemorrhage.[19–24] NOS inhibition also successfully and rapidly elevates blood pressure and systemic vascular resistance in septic shock patients.[25–28]

The first studies on NOS inhibition immediately triggered great hopes for a new treatment of refractory hypotension in (septic) shock, but even the earliest studies already indicated the potential harm of NOS inhibitors, as they also caused a progressive fall in cardiac output, amplified organ dysfunction, and even increased mortality.[26,29–33] Exacerbated organ damage was first reported for the kidney,[31] but later studies revealed increased injury in other organs as well, including liver, lung, pancreas, and intestines.[18] Unfortunately, even a phase III clinical trial had to be prematurely terminated because of increased mortality in the septic patients treated with the NOS inhibitor, despite positive effects on blood pressure and vascular resistance.[28] Together, these observations clearly indicate that NO not only mediates hypotension in septic shock, but may also perform an important obligatory role in assorted beneficial pathways.

NO IN SEPTIC SHOCK: DETRIMENTAL VERSUS BENEFICIAL EFFECTS

Different explanations may be suggested for the dual personality of NO during septic shock. First of all, there is no doubt about the detrimental effect of excessive NO on vasorelaxation, hypotension, and shock. The NO-mediated hypotension leads to severe hypoxia in peripheral vital organs, resulting in progressive organ failure. NO may also directly contribute to tissue and organ injury by its direct, peroxynitrite-mediated cytotoxic effects. It is generally accepted that NO may cause blood vessel relaxation by activating the cyclic guanosine monophosphate (cGMP)-producing enzyme soluble guanylate cyclase (sGC), leading to activation of the cGMP-dependent protein kinases (PKGs). For smooth muscle contraction, calcium-dependent activation of the myosin light chain (MLC) kinase and subsequent phosphorylation of MLC are essential. Several PKG-dependent phosphorylations ultimately converge on the dephosphorylation of MLC and hence relaxation[34] (Figure 1). Important molecular targets of PKG include various pumps and channels involved in modulating intracellular calcium levels and membrane potential, leading to decreased cytosolic calcium and relaxation. In addition to changes in intracellular calcium levels and membrane potential, other important targets for PKG in smooth muscle are the pathways regulating the calcium-sensitivity of the contractile machinery, more particularly the regulatory subunit of MLC phosphatase, which may be directly activated by PKG or indirectly via PKG-mediated inactivation of the inhibitory RhoA pathway.[35,36] Nevertheless, NO may also contribute independently of sGC and PKG to lower cytosolic calcium levels, for instance via direct S-nitrosation of potassium channels,[37] via NO-dependent peroxynitrite-mediated S-glutathiolation of the sarco/endoplasmic reticulum calcium adenosine triphosphatase (ATPase) (SERCA) pump,[38] or via direct inhibition of cytochrome P450 (CYP). Enzymes of the CYP4A family are known to produce the vasoconstrictor 20-HETE, an inhibitor of BK channels.[39] Although sGC has long been regarded as the predominant target for NO in the vasculature, the notion and importance of sGC-independent actions has gained considerable interest lately. The sGC-independent pathways would be especially important in certain vascular beds (particularly in the renal and mesenteric