

Nitric Oxide Donors in Endotoxic and Septic Shock: Evidence Against Nitric Oxide as a Mediator of Shock

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Although nitric oxide (NO) has been postulated to be a mediator of endotoxic or septic shock, a significant body of evidence has accumulated that is inconsistent with this notion. Therefore, the purpose of this chapter will be to present evidence showing that not only is there a reasonable doubt about many of the tenets underpinning the NO hypothesis, but that considerable data exist showing that NO actually counteracts the pathophysiology of shock states.

I will discuss five key suppositions regarding the role of nitric oxide as a key mediator of endotoxic or septic shock. These five suppositions are:

1. Nitric oxide levels reach such high values that they exert a constellation of effects which produce and promote shock states including septic shock.
2. Nitric oxide markedly depresses myocardial contractility, a hallmark of endotoxic or septic shock.
3. Nitric oxide synthase (NOS) inhibitors counteract the hemodynamic sequelae of endotoxic shock and improve survival in this state.
4. Nitric oxide donors aggravate endotoxic or septic shock.
5. Peroxynitrite, not nitric oxide, is the real culprit mediating shock states.

I will present significant evidence against each of these five major suppositions, which when taken together will cast significant doubt on the overall hypothesis that NO is indeed the mediator of endotoxic or septic shock.

1. Does nitric oxide increase to such high levels whereby it exerts significant effects that mediate endotoxic or septic shock?

Firstly, NO at physiological concentrations (i.e., 1-20 nM) exerts several important anti-shock effects which include (a) vasodilation [1], (b) inhibition of platelet aggregation [2], (c) attenuation of leukocyte adherence to the vascular endothelium [3,4,5], and (d) quenching of superoxide radicals [6,7]. All these effects are important actions of NO necessary for the maintenance of vascular and microcirculatory homeostasis. The argument is made that NO levels in endotoxic shock are so

high, that the vasodilator effects of NO spill over into the systemic circulation and produce profound systemic hypotension [8]. However, no evidence exists that these high levels of NO occur in the circulation or correlate with either hypotension or lethality [9].

Secondly, the generally accepted supposition is that NO levels in endotoxemic shock exist in the high micromolar range, but this has never been measured. In fact, the converse appears to be true, namely that endothelium-derived NO (EDNO) is markedly decreased in endotoxemia. Parker and Adams [10] have shown reduced EDNO in guinea pig aortic and coronary artery rings, and others have obtained similar results in rat mesenteric arteries [11], in dog coronary [12], or mesenteric arteries [13], and in sheep pulmonary arteries [14]. Moreover, Myers et al [15] demonstrated that both EDRF activity and NO production are directly inhibited by *E. coli* endotoxin in cultured endothelial cells. Moreover, blood vessels isolated from endotoxemic animals respond to NO releasing agents (e.g., nitroglycerin and NaNO₂), so that it is not a tachyphylaxis to NO that is responsible for the reduced responses to endothelium-dependent dilators.

Thirdly, endotoxin is thought to exert many of its effects via release of cytokines like tumor necrosis factor- α (TNF α). However, TNF α itself has been shown to reduce NO release (i.e., impair vasorelaxation to acetylcholine but not to direct vasodilators) in cat carotid arteries [16]. Furthermore, Preiser et al [17] failed to find evidence that NO mediated endotoxemic shock in dogs questioning whether high NO levels occur in endotoxic shock.

If, in fact, high circulating levels of NO are found in endotoxic or septic shock, and this remains an open question, then it is presumably the inducible form of NOS which triggers the elevated NO levels. Since this isoform of NOS occurs in macrophages at the site of the septic focus, the NO formed may not reach the sys-

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temic circulation. The elevated local NO may even be useful as an anti-bacterial agent. Indeed, it is this antimicrobial action which was one of the primary clues responsible for the discovery of NO [18]. In this regard, mutant mice lacking the iNOS gene are not resistant to endotoxin induced lethality [19].

Therefore, there is reasonable doubt that: (a) toxic levels of NO exist in endotoxic or septic shock, (b) iNOS activity solely contributes to the lethality of endotoxic or septic shock, and (c) elevated circulating levels of NO can overcome the loss of endothelium-derived NO observed in endotoxic shock.

2. Does nitric oxide exert a profound depression of cardiac contractility in endotoxic or septic shock?

This is one of the great myths regarding the role of NO in endotoxic shock. The concept was initiated by Finkel et al [20], who reported that L-arginine at 50–100 mM resulted in a 80–90% decrease in contractile force in isolated electrically stimulated hamster papillary muscles. This interesting report stimulated widespread interest in this alleged effect of NO. Unfortunately, it has not been independently confirmed, and may represent an artifact of arginine since D-arginine which also depresses cardiac muscle at 50–100 mM, was not used as a control. Weyrich et al [21] clearly showed that authentic NO gas dissolved in physiological solutions at concentrations which completely relax cat coronary arteries fail to exert any detectable inotropic effect in right ventricular papillary muscles from the same cat hearts. Moreover, a wide variety of NO donors (e.g., SNAP, cysteine NO donors, sydnonimines etc.) similarly fail to depress myocardial contractility at concentrations up to 100 μ M [21]. This lack of a negative inotropic effect of NO has been confirmed in the intact animal by Crystals [22] and by Klabundes groups [23]. Small negative inotropic effects of NO donors have been reported in isolated cardiomyocytes [21,24,25], but this usually occurs only under strong β -adrenergic stimulation. Biochemically, it is unlikely that a marked negative inotropic effect of NO can be observed, since cardiac myocytes contain large amounts of myoglobin, which acts as a nitric oxide sink and would most likely remove most of the NO before it could exert any cardiodepressant effect. To complicate matters even more, low concentrations of NO donors have been shown to increase contractility of isolated rat cardiac myocytes [26] and blockade of NOS increases cardiac contractility *in vivo* in dogs [27,23].

Recently, inhibition of nitric oxide biosynthesis has been shown to result in acute myocardial ischemia in endotoxemic rats suggesting that NO is necessary for normal cardiac integrity in shock [28]. Moreover, Meng et al [29] has recently shown that inhibition of NOS with L-NMMA failed to prevent cardiac contractile dysfunction in endotoxemic rats. These workers further concluded that the cardiac depression observed in

endotoxemia may not involve NO, and that inhibition of NOS may deteriorate coronary perfusion in endotoxemic hearts. This is also consistent with the results of Decking et al [30] in guinea pig cardiac myocytes isolated from endotoxemic guinea pigs. Clearly, further investigation is necessary to clarify these relationships. However, at this time no convincing data exist to substantiate the claim that NO markedly depresses cardiac contractility in endotoxic or septic shock, despite the early attractiveness of this hypothesis. Rather, NO may be necessary for normal cardiac and coronary vascular function.

3. Do nitric oxide synthase (NOS) inhibitors reverse the hemodynamic sequelae of endotoxic shock and improve survival in this situation?

One of the main tenets of the hypothesis that NO is a key mediator of endotoxic or septic shock is the postulate that inhibition of nitric oxide synthases are beneficial in these shock states. This subject has received widespread attention by many investigators since the highly interesting report of Petros et al [31] in 1991. This group was the first to treat patients in septic shock with a NOS inhibitor. Of the two shock patients receiving L-NMMA, one lived and one died, hardly a ringing endorsement of NOS inhibitor therapy in septic shock. Kilbourn [32] has championed the hypothesis that inhibition of NOS is a potentially important treatment in endotoxemic or septic shock, although Moncada's group [33] cautioned that inhibition of both constitutive and inducible NOS during endotoxemia is deleterious.

Recently, Cobb and Danner [34] have reviewed the endotoxic shock literature related to the effects of NOS inhibitors on hemodynamics and survival in large animals and humans. Of these 26 animal and four human studies, only five studies showed survival (i.e., dog and pig studies). Two of these studies showed reduced survival and three showed no change in survival rates. These results take on even greater significance since deleterious effects of NOS inhibitors contribute to increased mortality in endotoxemic rabbits [35] and endotoxemic mice [36].

Even more striking is the deleterious hemodynamic effects of NOS inhibitors (i.e., L-NAME, L-NMMA) in a wide variety of species including dogs, sheep, pigs and humans. Of the 28 studies in which systemic vascular resistance (SVR) was measured, 26 showed a marked increase in vascular resistance and two showed no change [34]. Coupled with cardiac output measurements in 29 studies, 22 showed a significant decrease and 7 exhibited no change in cardiac output during endotoxic shock. Noone observed an increase in cardiac output, an essential compensatory response in circulatory shock states like endotoxemic shock.

These latter findings point out the major flaw in the

hypothesis that NOS inhibition can protect in endotoxemic shock. This flaw is that by shutting off endogenous production of NO by the endothelium, one causes marked vasoconstriction, increasing arterial blood pressure at the expense of blood flow. NOS inhibitors also aggravate pulmonary hypertension and reduce oxygen delivery to the tissues. We should remember that circulatory shock is a consequence of a sustained and marked reduction in blood flow to the vital organs which if not reversed usually leads to cardiovascular collapse (i.e., circulatory shock) and eventually to death [37]. Thus, reducing cardiac output even further as is the case with NOS inhibitors, is counterproductive in endotoxemic shock.

In summary, there is reasonable doubt that NOS inhibition can protect in endotoxic shock. Moreover, it is very difficult to obtain a selective iNOS inhibitor that does not also inhibit eNOS. The selectivity is blurred at doses that are necessary to dramatically inhibit iNOS. In fact, the most recent class of "selective" iNOS inhibitors is cardiotoxic and had to be discontinued as potential therapeutic candidates [38]. Moreover, all NOS inhibitors when they block NO production also expose the host to latent virus infections which can be extremely dangerous and lethal [39] since NO functions normally to attenuate viral infections.

4. What effects do nitric oxide donors or nitric oxide itself have in endotoxic or septic shock?

Since endothelial NO production is reduced in endotoxemia (see section 1), and since endothelially derived nitric oxide exerts a variety of beneficial effects which are vasculoprotective in nature (see section 1), authentic NO gas or NO donors (e.g., organic NO donating compounds, usually nitrites or nitrates), have been studied in endotoxic and related forms of circulatory shock.

NO gas inhaled at low concentrations (i.e., 10-20 ppm) improved arterial PO_2 and decreased pulmonary artery pressure in patients with adult respiratory distress syndrome (ARDS), some of whom were septic [40]. These effects suggest that low NO concentrations may exert beneficial effects on pulmonary hemodynamics in sepsis. NO gas can also be dissolved in saline and infused locally into animals during shock states. In bowel ischemia shock, NO dissolved in solution and administered intravascularly to the splanchnic circulation improved the biochemical sequelae of bowel ischemia shock and markedly attenuated the formation of myocardial depressant factor (MDF) formed by the ischemic pancreas [41,42]. This same approach using dissolved NO infused locally worked in acute myocardial ischemia-reperfusion in cats [43].

Since NO gas is difficult to work with and requires special precautions, organic nitrates or nitrites which release NO in solution have been studied in life threatening emergencies. One such NO donor is S-nitroso-N-

acetylpenicillamine (SNAP) which is a very effective NO donating compound [44]. At low doses, SNAP is a vasodilator, and inhibits adherence of neutrophils to the vascular endothelium [45]. Furthermore, SNAP significantly attenuated endotoxin induced intestinal injury and plasma leakiness in rats [46]. Similar findings were observed in the endotoxemic liver under conditions where L-NMMA exacerbated hepatocellular injury in mice. This injury to the liver is reduced by administration of L-arginine, the precursor of NO biosynthesis [47,48]. Along these lines, endogenously synthesized NO has been shown to prevent endotoxin induced glomerular thrombosis in rats [49]. These findings were extended in endotoxemic rabbits to include improved hemodynamic effects [33]. Similar findings were observed in rats subjected to hemorrhagic shock, where SNAP improved hemodynamics, preserved the vascular endothelium, and extended survival time [50].

In acute intestinal inflammatory states, inhibition of NO synthesis aggravates intestinal injury [52]. Not surprisingly, another NO donor (i.e., C87-3754, a sydnonimine class NO donor) exerted significant endothelial preservation and beneficial hemodynamic effects while improving overall survival in cats subjected to bowel ischemia-reperfusion shock [53]. This NO donor as well as a novel cysteine NO donor were also cardioprotective in myocardial ischemia-reperfusion in cats [54,55]. Thus NO donors exert broad based cytoprotective effects in a variety of forms of ischemia and circulatory shock. These effects are not consistent with NO being the toxic mediator of endotoxin shock.

5. Does peroxynitrite (ONOO⁻) mediate toxic actions of NO in endotoxic or septic shock?

One of the most interesting hypothesis advanced to explain the alleged toxic effect of nitric oxide has been the "peroxynitrite hypotheses" proposed by Beckman and colleagues [56]. This hypothesis is based on the finding that NO and superoxide radical combine stoichiometrically at equimolar concentrations to produce peroxynitrite ONOO⁻. Purportedly, endotoxin can enhance ONOO⁻ formation by upregulating the inducible NOS [57]. The elevated ONOO⁻ can then activate poly-ADP ribosyltransferase (PARS) which causes DNA strand breaks, depletion of NAD⁺, and inhibition of mitochondrial respiration leading to vascular paralysis [58]. At high concentrations, peroxynitrite also causes lipid peroxidation of cell membranes, thus damaging cellular integrity [59].

However, there are several serious problems in the application of these concepts to mediating lethality in endotoxic or other forms of circulatory shock. Firstly, the cytotoxic effects of ONOO⁻ occur at the high micromolar to low millimolar concentration range (i.e., 500 μ M to 1.5 mM) [58,59]. It is highly unlikely that these

concentrations could ever occur in vivo since superoxide [60] and NO levels are 1–20 nM under normal conditions (1) and could realistically only increase by about two orders of magnitude to 1–10 μM , particularly since the half-life of ONOO⁻ is less than 1 second [60].

Secondly, much of the evidence that ONOO⁻ occurs in shock is indirect and based on the presence of nitrotyrosine, the so-called “footprint” of ONOO⁻ in biological systems [60,61]. However, recently nitrotyrosine has been shown not to be specific for ONOO⁻, since both cis- and trans-chlorine nitrite and other nitrites react to form nitrotyrosine [62]. Thus, much of the evidence that ONOO⁻ is present in tissues during shock must be reevaluated.

Thirdly, ONOO⁻ at physiologically relevant concentrations (i.e., 100–800 nM), actually exerts anti-shock activities including vasorelaxation and attenuation of leukocyte-endothelial interaction [63]. These effects translate into a marked protection of the isolated ischemic rat heart reperfused with neutrophils [63]. This protective effect is largely due to the down-regulation of P-selectin on the vascular endothelium. Consequently, neutrophil-induced cardiac dysfunction and myocyte injury are prevented. This cardioprotection has recently been extended to the intact cat subjected to myocardial ischemia/reperfusion and infused with μM peroxynitrite [64]. These important new findings suggest that physiological concentrations of ONOO⁻ may actually be beneficial in shock states, and casts very serious doubts that ONOO⁻ can be a significant mediator of cell injury in circulatory shock.

In summary, there is reasonable doubt regarding the validity of five of the major pillars of the NO mediation of endotoxic shock hypothesis. Moreover, in some cases, there is a significant body of evidence that the opposite may be true under certain circumstances (e.g., NO gas or NO donors at low concentrations) may actually exert significant anti-shock actions. Therefore, there is a need for additional carefully controlled studies to resolve this important question.

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