

## Is There NO Treatment For Severe Sepsis?

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### Abstract

Sepsis is a systemic inflammatory response syndrome in the presence of suspected or proven infection, and it may progress to or encompass organ failure (severe sepsis) and hypotension (septic shock). Clinicians possess an arsenal of supportive measures to combat severe sepsis and septic shock, and some success, albeit controversial, has been achieved by using low doses of corticosteroids or recombinant human activated protein C. However, a truly effective mediator-directed specific treatment has not been developed yet. Treatment with low doses of corticosteroids or with recombinant human activated protein C remains controversial and its success very limited. Attempts to treat shock by blocking LPS, TNF or IL-1 were unsuccessful, as were attempts to use interferon-gamma or granulocyte colony stimulating factor. Inhibiting nitric oxide synthases held promise but met with considerable difficulties. Scavenging excess nitric oxide or targeting molecules downstream of inducible nitric oxide synthase, such as soluble guanylate cyclase or potassium channels, might offer other alternatives.

Key words: sepsis, NOS, nitric oxide, corticosteroids, protein C

### Introduction

Sepsis may be defined as a systemic inflammatory response syndrome in the presence of suspected or proven infection. It is classified as severe sepsis if there is organ dysfunction, and as septic shock if severe shock is associated with hypotension despite fluid resuscitation. Sepsis is initiated by severe infections and precipitated by interactions between the pathogenic organism, the host immune and inflammatory responses, and coagulation processes [1]. The mortality rate from severe sepsis is 25-30% [2] and from septic shock it is 40-70% [3]. Though the rate of mortality due to septic shock has declined from 62% in the first part of the twentieth century to 56% by the year 2000 [4], its incidence has been rising [5,6]. Factors that could contribute to this rise include the increase in life expectancy and in the number of immunocompromised individuals, the more widespread use of invasive medical procedures and immunosuppressive therapy, the increase in microbial resistance, and the rising incidence of infection due to organisms other than bacteria. Though part of the reported increase in septic shock could be an artifact of the improvement in recognition and recording of the condition [7,8], mortality rates remain unacceptably high. Despite intense research, progress in therapy has clearly been inadequate, and it is for good reason that clinical trials on sepsis have been called "the graveyard of pharmaceutical companies." An arsenal of supportive measures is used to treat septic shock, but specific treatment that targets mediators of shock relies mostly on corticosteroids and recombinant human activated protein C [9,10].

### Mediator-directed treatment

Because a runaway inflammatory response is a major aspect of sepsis, most prospective therapies targeted mediators of inflammation. However, most strategies failed to improve survival in clinical trials, as described in a review [11]. One of the earliest therapeutic targets was the endotoxin of Gram-negative bacteria (LPS), but clinical trials employing blockade of LPS with specific antibodies failed to show significant benefit [12]. Another approach

tried to capitalize on the anti-inflammatory properties of corticosteroids by administering large doses to counteract the runaway immune responses [13], but a later study failed to demonstrate significant benefits for this approach [14]. By contrast, prolonged administration of low doses of corticosteroids as a hormonal replacement therapy to compensate for the lowered level of cortisol in many sepsis patients were recently shown to be beneficial [5].

Another well-known target is the potent proinflammatory cytokine, TNF, which is elevated in sepsis. Though animal experiments were promising, clinical trials failed to show any benefit for this approach [15,16]. Blocking IL-1 suffered a similar fate [17]. Attempts to reverse the immune suppression that occurs in sepsis by using interferon (IFN)- $\gamma$  or granulocyte colony stimulating factor (G-CSF) were also unsuccessful [18]. Yet another approach targeted the crosstalk between the coagulation and inflammatory systems; however, clinical trials using TF (tissue factor) antagonists, tissue factor-pathway inhibitor (TFPI) [19,20], or antithrombin (AT)-III [21] could not demonstrate significant benefit.

### Corticosteroids

The anti-inflammatory and hemodynamic effects of corticosteroids (glucocorticoids) have been known for a long time. They inhibit the production of inflammatory cytokines, prostaglandins, leukotrienes, and nitric oxide. Indeed, one of the homeostatic physiologic responses to sepsis is an increased level of stress hormones such as cortisol (though reduced responsiveness to corticotropin is also frequent). However, a meta-analysis of clinical trials showed that large doses of corticosteroids, despite their strong anti-inflammatory action, do not improve survival but may actually be harmful [22]. From these findings, and from the knowledge that adrenal insufficiency is part of sepsis, developed the notion of using physiologic doses of corticosteroids as adrenal replacement therapy in infection, sepsis, and septic shock.

A systematic review of clinical trials [5] concluded that long courses of low dose corticosteroids reduce mortality.

Another systematic study [23] recommended low dose corticosteroids for septic shock and counseled against high doses, except perhaps in some specific conditions; corticosteroids were not recommended for sepsis in the absence of shock. It is noteworthy that corticosteroid treatment was shown to be more beneficial for the more severely ill patients [24]. However, the use of corticosteroids in shock remained controversial [11]. The results of the recent CORTICUS trial, an international, prospective, randomized, double-blind, placebo controlled study, concluded that corticosteroid treatment did not reduce mortality, irrespective of ACTH responsiveness [25]. Though that report stated that the use of hydrocortisone was not associated with a higher incidence of superinfection, a more recent examination has revealed more superinfections and new sepsis and septic shock in steroid treated patients (Charles Sprung, personal communication).

### Activated protein C

Pro-inflammatory cytokines released in response to infection can initiate coagulation by activating tissue factor. Thrombin, which converts fibrinogen to fibrin and performs other functions in the coagulation process, can stimulate several inflammatory pathways, and moreover, coagulation and inflammation reciprocally amplify each other [26], potentially leading to organ failure.

Protein C is an important physiological anticoagulant [27]. Once activated by the thrombin-thrombomodulin complex, it acts to inhibit blood coagulation by proteolytically inactivating factors Va and VIIIa [28]. Moreover, activated protein C (APC) inhibits inflammatory cytokine production and limits the rolling of monocytes and neutrophils on injured endothelium [29]. APC is downregulated in sepsis, [30], which implies that it could be a useful treatment.

One landmark in the quest for a treatment for severe sepsis was a randomized, double-blind, placebo-controlled, multicenter trial, known as the PROWESS trial, conducted on 1690 sepsis patients who were at high risk of death [2]. This trial showed that rhAPC (recombinant human APC) reduces the absolute risk of death by 6.1%, and that although treatment was associated with a greater risk of bleeding, the benefits outweigh the increased bleeding risks. This study, however, has been criticized on methodological grounds [31,32]; the efficacy of rhAPC and the risk of bleeding have also been questioned [31-33]. Quite likely it is for these reasons that treatment with rhAPC has not been widely adopted by physicians [10], not to mention its extremely high cost [10,11]. Noteworthy is that rhAPC should not be used for adults with a low risk of death [34], and a recent, large, randomized, placebo-controlled study showed no benefit for rhAPC in children [35]. A very recent analysis [36] of several clinical trials on rhAPC did not come out in support of this treatment. It concluded that even in severely ill adult sepsis patients at high risk of death, for whom this treatment is approved, there is only weak evidence to support its use.

Despite this controversy, rhAPC remains on the table, and an animal study published during the preparation of this manuscript gave indications that showed that the

bleeding risk associated with this therapy may be overcome. By using a variant of APC with greatly reduced anticoagulant properties in a mouse model of sepsis, mortality was reduced without increasing the risk of bleeding [37]. Further research is needed to determine whether this strategy would be effective in humans.

### Targeting nitric oxide synthases

Following the discovery that nitric oxide is an important endogenous regulator of vascular tone [38-40], its importance in inflammatory and septic shock became evident. This highly reactive radical is produced by three different nitric oxide synthases (NOS). Neuronal nitric oxide (nNOS) and endothelial nitric oxide (eNOS) are constitutive enzymes that function in homeostatic processes such as neurotransmission and vascular tone, respectively, by producing small amounts of NO in response to increases in intracellular calcium. In contrast, iNOS is an inducible enzyme that is usually synthesized only in response to inflammation. Unlike the other two NOS, iNOS produces large amounts of NO for long periods of time [41].

Because NO is produced from L-arginine, its production can be inhibited by competitive L-arginine analogues, such as NG-monomethyl-L-arginine (L-NMMA), NG-nitro-L-arginine (L-NNA) and NG-nitro-L-arginine methyl ester (L-NAME). NOS inhibitors can prevent, revert, or at least minimize hypotension in shock induced by LPS, TNF, IL-1, IL-2 or hemorrhage [42-47]. NOS inhibition also elevates blood pressure and systemic vascular resistance in septic shock patients [48-51]. But NOS inhibition has limited therapeutic potential because it is associated with a progressive fall in cardiac output, amplified organ dysfunction, and even increased mortality [51-56]. In one phase III clinical trial, NOS inhibition increased mortality in septic patients despite its beneficial effects on blood pressure and vascular resistance [49]. General inhibition of NOS could be inappropriate for treatment of septic shock because eNOS might provide some protection against shock. This is clearly shown by the finding that transgenic expression of eNOS can partially protect mice against endotoxemia and polymicrobial sepsis [57,58]. This dichotomous effect of NO places a hurdle in the way of developing NOS inhibitors as a treatment for shock, and so attempts were made to overcome it by using specific iNOS inhibitors.

The effects of specific iNOS inhibition on organ function are somewhat controversial, but it does not seem to lead to deleterious effects of the same degree as those caused by general NOS inhibition. Moreover, iNOS inhibition prevented or reverted circulatory failure in all the reports [59]. Unfortunately, though iNOS inhibition seemed a promising therapeutic strategy, experiments on iNOS-deficient mice indicated otherwise. Not only were these mice not protected against endotoxemia, sepsis or TNF-induced shock, they even suffered higher mortality rates in some studies [60-64].

Clearly, iNOS inhibitors and iNOS deficiency do not have the same effects. It is conceivable that during iNOS inhibition the anti-apoptotic or anti-oxidative effects of some residual NO may provide some benefit [65,66]. Alternatively, iNOS inhibitors could have additional

pharmacological effects unrelated to iNOS inhibition, as exemplified by the anti-oxidative effects of S-methylisothiourea [67], and the inhibition of catalase by aminoguanidine [68], both of which are iNOS inhibitors.

Simple specific iNOS inhibition does not seem to be a valid approach to treating shock, because it does not always diminish organ damage or mortality in experimental endotoxic or septic shock. Thus it would be more reasonable to selectively modulate the downstream targets of NO that play important roles in the hypotensive effects of NO.

### Scavenging NO

An alternative to inhibiting iNOS, especially that no specific inhibitor for it is available, is to scavenge excess NO. This could also have the advantage of preserving some NO at its production locations, where it can perform normal physiological functions. Several NO scavengers have been evaluated with some positive results in different animal models of shock [69-72]. One interesting NO scavenger, pyridoxalated hemoglobin polyoxyethylene (PHP), has been evaluated in distributive shock. Because the study recruited SIRS patients while excluding those with significant cardiac disease, burns or trauma, and those who were at risk of dying shortly from an underlying condition, the results are relevant to sepsis. In that study, PHP raised blood pressure and reduced vasopressor and ventilation needs without causing organ damage or adversely effecting cardiac output or survival [73]. PHP has entered phase III clinical trials and the results are expected to be published soon (Gary Kinasewitz, personal communication).

### Targets downstream of iNOS

#### Soluble guanylate cyclase (sGC)

Binding of NO to sGC, considered its main cardiovascular "receptor", leads to accumulation of cGMP, which in turn leads to vascular relaxation, myocardial depression, and inhibition of platelet aggregation and adhesion [74]. This indicates that the cGMP pathway might be a potential target for treatment of shock. Inhibiting cGMP production with methylene blue (MB) protects mice against experimental shock induced by TNF [61], but not against endotoxemia [75]. Analogously, infusion of MB in humans suffering septic shock reverses hypotension, but does not change the overall mortality rate [76-79]. It has been speculated that its protective effects could be due to effects on oxidative stress that are not sGC-dependent [65].

#### K<sup>+</sup> channels

K<sup>+</sup> channels play an important role in regulating membrane potential, and thus hyperpolarization and relaxation of vascular smooth muscle cells. Because NO may activate K<sup>+</sup> channels both sGC-dependently and sGC-independently [80-83], inhibition of K<sup>+</sup> channels might offer an alternative strategy for treatment of shock.

In the vasculature, the most important K<sup>+</sup> channel subclasses that are involved in the action of various endothelium-derived relaxing factors are the ATP-sensitive KATP channel and the large conductance calcium-activated BK channel. KATP channels have long been

suspected of playing the most important role in septic shock [84,85]. They are activated by decreased ATP, increased lactate, and acidosis, all of which characterize sepsis. In addition, they may also be activated by NO, prostacyclin (PGI<sub>2</sub>), and the recently identified vasodilator, H<sub>2</sub>S [83,84,86]. In many endotoxic animal models, inhibiting KATP by parenteral glibenclamide could partially return hypotension and vascular hyporesponsiveness to normal [87-90]. However, glibenclamide did not restore responsiveness in another animal study [91], or in a recent clinical trial [92]. Failure in the clinical trial might have been due to administration of the drug by the enteric route, or to the mild lactic acidosis in the enrolled patients [93]. In any event, these results indicate that KATP inhibition might not be the solution for septic shock.

BK channels are probably the most important channels involved in NO-dependent vascular relaxation [82,83]. NO may activate BK channels sGC-dependently through phosphorylation by cGMP-dependent protein kinase, as well as directly via S-nitrosation without requiring cGMP [81,94,95]. BK channels are also targeted by other potential vasodilators, including H<sub>2</sub>O<sub>2</sub> and epoxyeicosatrienoic acids (EETs) [96-98]. These results indicate that inhibiting BK channels could be useful in treatment of shock. Unfortunately, most of the few published animal studies have not used specific BK-inhibitors, but rather tetraethylammonium (TEA), a non-specific inhibitor of BK, KATP and certain voltage-gated KV channels. Though TEA neither improved blood pressure nor decreased mortality in one animal study [99], it successfully restored vascular responsiveness in another [91], and in an experimental human endotoxemia study [100]. But very recently Cauwels et al., using various inhibitors, including iberiotoxin and apamin, which are specific for BK channels and small conductance calcium-dependent SK channels, respectively, showed that both of these channels are involved in mouse models of TNF- and LPS-induced shock [75]. This indicates that these channels could be potential targets for treatment of septic shock.

### Conclusions

Despite decades of research, there have been very few mediator-specific treatments that consistently improve survival of sepsis patients. Corticosteroids and activated protein C have been in clinical use and are claimed to save lives, but considerable controversy surrounds their efficacy and side effects. Clinicians still have to rely in most cases on conventional supportive measures to save patients' lives. High hopes were set for NOS inhibition, but this approach was not successful. Inhibition of iNOS may provide better results, but more specific iNOS inhibitors would be needed before this approach can be tested in clinical trials. Alternatively, NO scavengers such as PHP could be considered for more testing in animals and in human trials as scavengers of excessive NO. Otherwise, focus can be shifted to downstream targets of NO instead of trying to interfere with production of NO, which is clearly a Janus-faced molecule in septic shock.

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