Review

Effects of endothelin and nitric oxide on organ injury, mesenteric ischemia, and survival in experimental models of septic shock

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ABSTRACT

The development of potent drugs to treat cardiopulmonary failure in sepsis, such as antibiotics and new immunomodulatory therapeutic approaches have not prevented sepsis from being a major health problem. Dysfunction of the vascular endothelium is an early event in septic shock. The recognition of endothelium-derived substances, such as nitric oxide and endothelin, important mediators of systemic inflammatory response syndrome, led to the proposal that pharmacological inhibition of nitric oxide and endothelin production could represent a useful strategy in the treatment of septic shock.

Splanchnic ischemia and translocation of endotoxin from the gut to the circulation contributes significantly to the high mortality rate in sepsis-related syndromes. This vasoconstriction in the splanchnic circulation can be partially blocked by inducible nitric oxide synthase inhibitor aminoguanidine or endothelin receptor antagonist bosentan in experimental models of septic shock.

It can be suggested that endothelin and nitric oxide may affect survival. Although septic shock is a highly complex pathophysiological state, the course of septic shock has different phases with different characteristics which need different (special) treatment strategy. The inhibition of nitric oxide production during hyperdynamic, earlier phase of sepsis combined with the blockade of endothelin receptors at a later stage during the hypodynamic, late phase appears to be a novel promising strategy for the therapy of septic shock.

The aim of this review is to discuss the role of nitric oxide and endothelin in sepsis and the potential therapeutic implications of blockade of nitric oxide and endothelin as a target in treatment of human septic shock. Briefly the importance of timing of intervention is also emphasized.

SEPTIC SHOCK

Septic shock is a serious progressive failure of the circulation with high mortality rate of 30 %-90 %[1]. Although it is widely accepted that the course of septic shock has different phases with different characteristics[2], most of the therapeutical interventions are uniformly based on the principal aim of combating with the refractory hypotension by using aggressive fluid infusions, large doses of vasoconstrictors and glucocorticoids which do not offer consistent success[3].

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Current therapeutic approaches for septic shock also include antimicrobial chemotherapy, oxygen therapy, mechanical ventilation, and hemodialysis which failed to make substantial impact on the high mortality associated with septic shock[3]. Therefore, lessons learned from previous studies and failure of the current approaches should stimulate researchers to find new treatment motalities.

The concept of sepsis syndrome relies on the belief that the pathophysiolgy of septic shock is similiar whatever the microbial aetiology and primary focus of infection may be. The cascade of events that lead to the highly fatal outcome of septic shock is believed to be triggered by the entrance of mainly the Gram (-) endotoxin into the systemic circulation although other bioactive components like exotoxin, lipoteichoic acid, and peptidoglycan complexes can also produce a similar outcome including hypotension, microvascular injury, disseminated intravascular coagulation, and diminished blood flow to vital organs that lead to multiple organ failure[4].

SEPTIC SHOCK AND ENDOTHELIUM

The endothelium lining the circulatory system serves as an important target and a modulator for the effects of endotoxin because of its close contact with circulating blood and its proximity to the underlying vascular smooth muscle. Prolonged periods of septic shock result in the development of an endothelial dysfunction. Endothelium is now recognized as an endocrine/paracrine structure which secretes several vasoactive mediators or autacoids that decisively affect vascular tone and platelet function.

Endothelium-derived substances nitric oxide[5] and endothelin[6] are regarded as key mediators in systemic inflammatory response syndrome that lead to fatal multiple organ dysfunction[4] based on the outcome of animal experiments[7] and a limited number of clinical observations involving human subjects[8].

NITRIC OXIDE AND ENDOTHELIN: MORE THAN ENDOTHELIUM-DERIVED AGENTS IN SEPTIC SHOCK

Nitric oxide is believed to play a key role in the pathogenesis of septic shock. In several experimental models, endotoxin has been shown to increase the constitutive release of nitric oxide by the endothelium and the activity of iNOS[5]. Nitric oxide, produced at copious amounts by inducible nitric oxide synthase[9] may contribute significantly to the deleterious effects of endotoxin such as hypotension[10], vascular unresponsiveness (vasoplegia)[11,12], cardiodepression[1], and organ injury and dysfunction[7] in septic shock. The inhibition of both endothelial and inducible nitric oxide synthases by non-selective inhibitor *L*-NAME (N^G-substituted *L*-arginine analog) has been shown to exacerbate endotoxin-induced organ ischemia and accelerate death (adverse effects)[13]. More recently, attention has been focused on the inhibition of inducible nitric oxide synthase as this isoform is selectively increased during sepsis and has a greater significance. When this enzyme was blocked selectively by *L*-canavanine[14], the mice challenged with a lethal dose of endotoxin were reported to exhibit significant improvements in hemodynamic and metabolic parameters as well as increased survival[15]. Taken together, basal release of nitric oxide by endothelial nitric oxide synthase has an important role in the regulation of regional blood flow (beneficial effects), while the excessive generation of nitric oxide by inducible nitric oxide synthase inhibited regional blood flow (harmful effects).

The case for the role of endothelin(s) in sepsis is also similar. In particular, endothelin release is stimulated by endotoxin[16] and endothelin peptides are increased in the circulation of septic patients[17], which may be a beneficial effect in maintaining the blood pressure and organ perfusion during the early phase of septic shock[18]. On the other hand, excessive rise in the plasma level of endothelin for longer periods evokes profound vasoconstriction and tissue hypoperfusion in various vascular beds, which is indeed harmful[19].

ROLE OF NITRIC OXIDE AND ENDOTHELIN BLOCKAGE ON MULTIPLE ORGAN FAILURE, SPLANCHNIC HYPOPERFUSION AND BACTERIAL TRANSLOCATION IN SEPTIC SHOCK

It is widely accepted that endotoxin also produces multiple organ failure which is a frequent cause of death among patients who succumb to endotoxic shock. Based on our own data as well as results reported in the literature, endotoxin produces severe hemocongestion in the liver parenchyma, profound hydropic degeneration, inflammatory lymphocytic infiltration around the bile canaliculi together with the formation of minimal parenchymas injury in the form of spotty necrosis in liver[7]. When the animals were pretreated with

endothelin receptor antagonist bosentan, liver architecture was well preserved and the organs were completely protected from the histopathological injuries inflicted by endotoxin[7]. Interestingly, in contrast to the ineffectiveness of solitary usage of *L*-NAME, a relatively specific nitric oxide synthase inhibitor aminoguanidine also provided a significant protection against endotoxin-induced inflammatory injury[7]. In contrast to our results, MacMicking *et al* reported that although the hypotension and early mortality were reduced, there was no significant difference between the knockout mice deficient from inducible nitric oxide synthase and normal-wild type with regard to lipopolysaccharide/ *Clostridium parvum*-induced hepatic injury and they concluded that nitric oxide was not the key mediator in septic shock[29]. The inhibition of histamine metabolism, polyamine catabolism, catalase activity, formation of glycosylation end-products or oxidative stress, and lipoperoxidation by aminoguanidine may help to explain our present findings[21]. To sum up the evidence gathered from experimental animal studies, no single agent can be implicated as the mediator of endotoxin-induced organ injury during sepsis[30].

It has been widely agreed that perfusion in splanchnic circulation decreases in advanced septic states. Septic shock is classified under the "distributive" type of circulatory disturbances or perfusion maldistributions which imply pooling of blood in some organs while some other vascular beds remain relatively ischemic[20]. Septic shock is characterized by refractory hypotension and profound vasodilation, but blood flow is diminished in some vascular beds such as splanchnic circulation, indicating that some vasoconstrictor mechanisms are activated in reponse to endotoxin[7, 21-23]. This vasoconstriction may explain the development of organ dysfunction, intestinal ischemia, and the related translocation of endotoxin from the gut to the circulation seen in sepsis syndrome. During the progression of sepsis, the intestinal mucosa functions as a major local defense barrier that helps to prevent dissemination of bacteria and endotoxin normally within the lumen. Endotoxemia-induced splanchnic ischemia is supposed to impair the barrier function of the gastrointestinal mucosa against the microorganisms resident in the lumen. Impaired perfusion of splanchnic organs may result exacerbations of polymicrobial bacteremia due to intestinal mucosal leakage[24]. As predicted, endotoxin administration results in the migration of viable bacteria into the mesenteric lymph nodes, liver, spleen, and peripheral circulation through portal portal circulation, ie, bacterial transclocation. Evaluation of the splanchnic vasoregulation and maintenance of perfusion is vital for the management of the septic patient. It is possible that the underlying mechanism involves the actions of endothelin-1 or nitric oxide which were shown to be released in sepsis. There is no clear agreement on the contributions of these substances in septic shock but endothelin, the most potent vasoconstrictor that is produced by the endothelium, appearing to be the most likely candidate for this vasoconstricton. Since splanchnic ischemia contributes significantly to the high mortality rate in sepsis-related syndromes[25], increasing blood flow to mesenteric circulation by blocking endothelin appears to be promising to prevente organ injury and treat septic shock. It is possible to abolish the deleterious effects of endotoxin such as mesenteric vasoconstriction[7] by using bosentan, a non-specific endothelin ET_A and endothelin ET_B receptor antagonist with no intrinsic agonist activity[26]. Improvement in the cardiovascular performance by increasing blood flow to the splanchnic and intestinal vascular beds due to endothelin blockade by using bosentan in a porcine model of septic shock[27] and another work in rats[28] revealed that vasoconstrictor mechanisms were indeed prominent in the splanchnic area during sepsis. Therefore the harmful effects of endotoxin in mesenteric vascular bed are mostly mediated by endothelin peptides rather than nitric oxide, and endothelin-mediated mechanisms play a significant role in splanchnic perfusion disturbances during sepsis. It remains unclear how this peptide is temporally released in the circulation in patients with septic shock and whether it has a predictive value with regard to mortality.

A NOVEL PERSPECTIVE FOR THE THERAPY OF SEPTIC SHOCK: NITRIC OXIDE AND ENDOTHELIN BLOCKADE

Independent from the type of initiating events, most of the patients that eventually die from septic shock exhibit similar clinical features which can be categorized into at least four distinct clinical phases, namely: preshock, hyperdynamic, hypodynamic, and irreversible phases[2]. Among these, the hyperdynamic phase and its follower, the hypodynamic phase, display markedly opposite characteristics with regard to the cardiovascular system parameters. During the hyperdynamic phase which is also named as "early", "warm", or

"compensated", the cardiac output is elevated and systemic vascular resistance is low[31] indicating the predominance of vasodilator mechanisms. Nitric oxide-related mechanisms appear to be one of the main role-player during this early phase. In contrast, during the hypodynamic, "late", "cold", or "decompensated" phase, the cardiac output is low and the systemic vascular resistance is increased[32] due to circulating vasoconstrictors accompanied by an increase in vascular permeability resulting from fluid flux into the extravascular space[33]. Endothelin-related mechanisms appear to be one of the main role-player during this late phase. Thus, these two potent vasoactive agents have divergent time course that are likely to be related to the different mechanisms of control of the vascular tone during sepsis. It seems that different agents are needed at different time during the septic process for treatment.

We have demonstrated that in a mouse model of septic shock with a rather high mortality rate of 90 % at 24 h, it was possible to impove the survival by inhibiting nitric oxide during the "early" stage combined with relatively "late" inhibition of endothelin under the endotoxin challenge[34]. In the same study inhibition of nitric oxide alone reversed sepsis-induced hypotension and early mortality, yet uniformly resulted in poor outcome. This result shows that blood pressure probably does not equate organ blood flow and perfusion. Although there are discordant results observed between animal models and clinical trials in sepsis, inhibition of nitric oxide during the "early" stage combined with relatively "late" inhibition of endothelin appears to be the correct approach in septic shock. The combination of both therapies in different time intervals is superior to either alone in improving survival.

CONCLUSION

According to our findings the timing of endothelin and nitric oxide inhibition may affect survival. As a first step toward evaluating this suggestion, the endothelin levels must be measured in septic patients. This will also provide insights for determining the timing of administration of endothelin antagonist and nitric oxide synthase blocker . We must also keep in mind that human sepsis has a time course that may be substantially longer than those of most experimental studies, which are conducted in a short-term perspective. Long-term studies of sepsis, utilizing endothelin and nitric oxide antagonists, are still lacking. Limited data are available concerning the actions of endothelin and nitric oxide in human septic shock. Therefore the need for a better knowledge of the timing of endothelin increase in humans is crucial if we are to make further progress in this field. With the recent failure in various clinical trials with antibodies against endotoxin or inflammatory cytokines, the most reasonable approach to the treatment of septic patients remains a combination of drugs against various key mediators, such as nitric oxide and endothelin, with particular emphasis on the timing of the administration of their blockers.

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