

Endotoxin in Parenteral Medicines may be a Risk Factor in Severe Sepsis

SUSAN SZATHMARY¹, EDIT HEGYI², and PETER GRANDICS^{1,*}

¹Clarigen, Inc. 5922 Farnsworth Ct. Carlsbad, CA 92008; ²Department of Pathology, UC Davis Medical Center, 4400 "V" Street, Sacramento, CA 95816

Introduction

Endotoxemia is part of sepsis and septic shock. In the terminal phase of the disease, elevated blood levels of endotoxin (ET) are commonly observed. This suggests a correlation between endotoxemia and the severity of sepsis. Proliferating microorganisms are the primary source of ET in septic patients; however, ET is also present in replacement fluids and in a variety of iv drugs and blood products. Current pharmacopoeia guidelines allow 0.5EU/ml in replacement fluids (1) and 350EU/dose (assuming 70 kg body weight) for any iv medication (2).

The ET binding and neutralizing function of the human organism involves a complex mechanism. Animal experiments show that the reticuloendothelial system (spleen, liver) plays an important role in this process (3, 4). Critically ill septic patients have a decreased tolerance for exogenous ET due to the fact that ET produced by the infectious agent already saturates most binding sites for ET. Our goal is to express concern and raise awareness about the importance of inadvertently administering ET to critically ill patients in their iv medications and replacement fluids. We have calculated how much ET could be contained by iv medications, replacement fluids, and blood products commonly given to septic patients with special attention to the burn patient population. We offer a warning regarding the potential harmful effects of ET in iv products.

Discussion

The presence of pyrogens in the blood is responsible for inflammatory reactions that can be fatal, if unchecked, as in the case of septic shock. Approximately 500,000 patients suffer from sepsis in the U.S. annually, and the mortality of patients with septic shock is reported to be 35-60% (5-7). This makes sepsis the 13th leading cause of death (8). Yet, decades of research to overcome septicemia or even significantly decrease its mortality rate have been unsuccessful.

Gram-negative bacterial infections account for a large fraction of sepsis cases. The pathogenesis of sepsis begins with the proliferation of microorganisms at a nidus of infection, followed by invasion of the bloodstream and organs. As a structural component of gram-negative bacteria, ET plays a pivotal role in the initiation and development of the sepsis syndrome. The most widely accepted theory of sepsis proposes that ET binds to the CD14 receptor, leading to the activation of monocytes and other cells carrying this receptor.

This binding results in transcriptional changes in the nucleus of activated cells, and the synthesis of inflammatory compounds including platelet activating factor-1 (PAF-I), pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF- α), and interleukin-1 (IL-1), IL-6, IL-8, and IL-12 that initiate, systemic inflammatory response. In the case of gram-positive or fungus-mediated sepsis, translocation of gram-negative bacteria (and consequently ET) from the gut into the circulatory system could also contribute to the development of septic shock (9).

Two lines of new evidence support the view that lipopolysaccharide (LPS) plays a pivotal role in the pathomechanism of sepsis. Dendritic cells exposed to purified LPS showed a nearly 88% replication of the gene transcript profile obtained when the cells were

* Author to whom correspondence should be addressed: Clarigen, Inc., 5922 Farnsworth Ct., Carlsbad, CA 92008, Phone: 760-929-4996, Fax: 760-9294982, E-mail: pgrandics@earthlink.net.

exposed to whole *E. coli* (10). Also, a lipid A-deficient strain of *Nisseria meningitides* induced 10-times less TNF when compared to the wild type strain (11). Experiments also showed that sepsis occurred after injecting endotoxin into animals or humans (12, 13).

Endotoxin can potentiate the effects of cytokines and mediators. A small amount of ET markedly increased the effects of TNF in a mouse model of septic shock (14). Also, the toxicity of toxic shock syndrome toxin (TSST-1) increased 50,000-fold in the presence of ET (15), indicating a mechanism that amplifies ET action.

The clearance of endotoxemia is influenced by a variety of ET and host-specific factors, including uptake into the liver, spleen, and phagocytic cells as well as humoral inactivation (16, 17). The dysfunction and saturation of the clearance system in severe sepsis is responsible for the exacerbation of endotoxemia and bacteremia. Evidence shows a link between endotoxemia and bacteremia including delayed clearance of bacteria in rabbits infused with ET (18). Patients with gram-negative bacteremia exhibit an association between endotoxemia, severity of disease, and low opsonizing antibody titers (19). Endotoxin measurements in blood samples seem to offer a prognostic value of endotoxemia with regard to mortality (20-22).

In studies of patients with burn injuries, endotoxemia correlates with the size of injury (23, 24), sepsis score (25), gram-negative bacteremia (26), and mortality (23, 27). Endotoxemia associated with the development of acute respiratory distress syndrome (ARDS) with sepsis patients has been reported (28, 29). Healthy volunteers administered with ET showed increased intestinal permeability (30), a phenomenon that may amplify endotoxemia and bacteremia in septic patients.

The promising results regarding extracorporeal ET removal with septic shock patients also underscores the significance of ET in the pathomechanism of septic shock. Most patients with endotoxemia and septic shock reported levels of ET in the range of < 120pg/ml plasma (31-33). Other authors using a different endotoxin standard and test kit reported a median serum value of 300pg/ml (34).

Survival of septic shock patients has improved significantly by removing 32-45% of the circulating ET (28-30). Interestingly, the day after the extracorporeal

therapy, circulating ET levels spontaneously dropped by 34% (31). This suggests that reducing the ET burden may help restore ET clearance mechanisms. Also, a relatively narrow margin (about 25%) was reported in initial plasma endotoxin levels between survivors and nonsurvivors (31). Based on measurements of circulating ET levels and clinical outcome, it seems that after reaching a threshold plasma ET level, the likelihood of recovering from endotoxemia and sepsis is low.

These observations teach us an important lesson about therapies for septic shock. Currently, the standard treatment remains an urgent stabilization of the patient, resuscitation and volume expansion, vasopressors and/or inotropic therapy to restore perfusion, and the administration of broad-spectrum antibiotic therapy in an ICU setting.

Depending on the percentage of skin area burned, during the first 24 h of volume resuscitation alone the burn patient may receive 10 L or more of sterile infusion fluid with an allowed maximal endotoxin content of 0.5EU/ml (50pg/ml). Volume-maintaining blood products and antibiotics can each introduce a maximum of 350EU (35 ng) of ET (2) into the patient's circulation (assuming a patient body weight of 70 kg). Adding up the numbers for legally allowed ET shows that burn patients could receive as much as 500 ng or more ET over a short period of time during the early phase of therapy. This is more than the total amount of circulating ET reported for patients in septic shock (31-34) and far exceeds the maximum allowed ET of 35 ng for a single dose of parenteral drug (2). Current ET standards have never addressed such situations, that is, what happens in the event of concurrent, multiple administration of parenterals as is common in the case of severe sepsis.

A very recent study has found that the use of serum albumin could be contraindicated for resuscitation and volume expansion of the critically ill (septic or burn) patients, as it increases the risk of death (35). This observation is intriguing as albumin actually participates in systemic detoxification *in vivo*. However, albumin is also known to be an avid binder and carrier for ET (36). Pyrogenic reactions to injectables made by parenteral manufacturers and compounding pharmacies (37-40) that were released within or near USP specifications suggested that trace contamination of non-ET pyrogens act synergistically with subpyrogenic levels of ET. These high-risk products with adventitious impurities are of

natural origin produced by fractionation methods, fermentation, or recombinant technology.

Testing for such impurities is not included in current release criteria for parenteral products. The severity of the symptoms of sepsis may well obscure potential adverse reactions to therapeutic agents.

The mortality rate is high in cases of septic shock. The fact that ET clearance is reduced in septic patients strongly indicates that, as in animal models (3, 4), a large proportion of ET is captured by the liver, spleen and lungs, saturating these organs with ET. In rabbits, there is a rapid clearance phase and a prolonged clearance phase following an iv ET challenge (4). By binding with ET, the liver, spleen, and lungs achieve rapid clearance during the initial period ($t_{1/2} < 30\text{min}$). In the second or prolonged phase, ET accumulates mostly in the adrenal glands, involving the clearance of low-density LPS.

When only low-density LPS was introduced into the circulation, the rapid phase failed to occur, indicating a different mechanism for the two phases of ET clearance. A certain amount of mixed-density LPS can be transformed into low-density ET in animals, but when the primary binding sites (liver, spleen, and lung macrophages) are saturated, and ET levels in the blood exceed the capacity of the clearance mechanism (both rapid and prolonged), the blood levels of ET rise in parallel with the introduction of new ET into the circulation. Accumulation of LPS in the adrenal gland may further compromise the survival of septic shock patients. This study suggests that the mechanism by which the molecular heterogeneity of LPS affects its organ distribution could influence clinical results.

These observations raise concern regarding the introduction of ET through the administration of parenteral medicines and fluids. In addition, it should be noted that the limit for the allowed ET per injection was determined from studies on healthy volunteers (41). Introducing such amounts of ET may be much more detrimental to septic patients.

Recent studies suggest that some patients, that is, those carrying a polymorphism within the coding region of the CD 14 receptor, have an altered response to ET that may further worsen the outcome of sepsis (42). Additionally, a specific polymorphism in the

TLR4 gene, that substantially alters host responses to Gram-negative pathogens, predominantly occurs in sepsis patients (43), suggesting that genetic factors may predispose some patients to an unfavorable clinical outcome.

This makes it even more important to revise the current ET standard. Clearly, it would be preferable to avoid the inclusion of any microbial components in parenteral products. Readily available technology would allow reduction in ET levels in parenterals (44-46) below current standards.

In a previous publication, we reviewed recently discovered pathological effects of low levels of ET administration and suggested that the ET level currently allowed in parenteral medicines may increase morbidity and mortality in patients treated for chronic diseases as well as those who are critically ill, e.g., patients in severe sepsis (47). This paper provides further data on this subject.

In conclusion, a review of available literature suggest that patients in septic shock could receive an amount of ET and potentially other yet-unidentified microbial components from parenteral medications comparable to or even higher than their own circulating ET burden. As endotoxemia correlates with clinical outcome in cases of septic shock, such inadvertent ET administration may increase the mortality of the critically ill.

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