REVIEW ARTICLE

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Current Trends and Future Implications in Sepsis Treatment

Abstract: Sepsis is a systemic illness caused by invasion of tissues by pathogens. It is highly lethal, causing circulatory failure and death. There has been extensive research trying to identify possible therapies for sepsis. Apart from source control, and antibiotic and supportive therapy, glucocorticoids, anticoagulant agents, and immunoglobulins have been tried in both the experimental setting and in septic patients. Newer strategies include novel anticoagulant agents like ethyl pyruvate, statins, insulin receptor modulators and Toll-like receptor modulators. This review article summarizes both the current therapies and endeavors for future treatment in sepsis.

Key Words: Sepsis, glucocorticoids, insulin, immunoglobulins, anticoagulants, Toll-like receptors

Sepsis Tedavisindeki Güncel Gelişmeler

Özet: Sepsis, dokuların patojen organizmalar tarafından işgal edilmesi sonucu ortaya çıkan sistemik bir hastalıktır. Dolaşım bozukluğu ve ölümle sonuçlanabilir. Sepsis tedavisi alanında birçok araştırma yapılmıştır. Kaynak kontrolü, antibiyotik ve destek tedavisi dışında glukokortikoidler, immunoglobulinler, antikoagülan ajanlar da hem deneysel hem de klinikte kullanılan tedavi yöntemleridir. Yeni tedavi stratejileri etil pirüvat gibi antikoagülanları, statinleri, insülin ve toll-like reseptör modülatörlerini içermektedir. Bu makalede sepsisin hem güncel tedavisi hem de gelecekte uygulanabilecek olası tedavi yaklaşımları tartışılmıştır.

Anahtar Sözcükler: Sepsis, glukokortikoidler, insülin, immunoglobulinler, antikoagülanlar, Toll-like reseptörler

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Introduction

For centuries, physicians have encountered the devastating consequences of infection in forms of circulatory collapse and death. The link between bloodstream infection and systemic illness is referred to as septicemia. Sepsis is a systemic illness caused by microbial invasion of parts of the human body during the course of septicemia. It is characterized by non-specific inflammatory responses with evidence of a microbial origin. If hypoperfusion or dysfunction of at least one organ system accompanies, the situation is called severe sepsis. Septic shock is diagnosed when severe sepsis is complicated by hypotension unresponsive to fluid resuscitation and vasopressor therapy becomes necessary (1).

The Centers for Disease Control (CDC) found the incidence of sepsis as 175.9 per 100,000 in 1989. This figure was found to be doubled when compared to 1979 owing to the increased prevalence of patients with human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS). The rates of sepsis in intensive care units are cited to be between 4.5% and 26% (2).

In hospitalized patients, sepsis is a major cause of morbidity and mortality. The mortality rate ranges between 30% and 50%, killing 250,000 patients every year in the United States (3). The classical methods of treatment include the maintenance of systemic perfusion and the eradication of infectious sources.

There has been extensive research in the use of alternative therapies directed at changing the disturbed host responses that occur during sepsis. In sepsis, infection rapidly activates systemic host defenses including the complement, coagulation and kallikrein-kinin systems as well as response of inflammatory cells, which results in amplification of the immune response with release of proinflammatory mediators. Platelet activation and microvascular thrombi formation induce localized tissue ischemia and contribute to the inflammatory response. Endogenous anti-inflammatory and anticoagulant factors are often insufficient to counteract the effects of widespread inflammation and tissue ischemia (4).

In the last decade, there has been a substantial effort to identify factors in the host response to infection that could be used for therapy. This review summarizes both classic and novel approaches in sepsis treatment, in chronological order.

Glucocorticoids:

Corticosteroids were proposed for the treatment of patients with severe sepsis as early as 1940 (5). This is based on the idea that septic shock may be complicated by an adrenal insufficiency and a peripheral glucocorticoid resistance syndrome. Diagnosis of adrenal insufficiency is based on a cortisol increase after a corticotropin test of less than 9 mg/dl. Using this definition, the prevalence of occult adrenal insufficiency in severe sepsis was estimated at about 50% and the 28-day mortality rate at about 75% (6).

In sepsis, the hypothalamic-pituitary adrenal axis is activated through systemic and neural pathways. Impaired adrenal function is responsible for the vessels' decreased sensitivity to norepinephrine in severe sepsis (7).

Cortisol inhibits the synthesis of various pro-inflammatory factors and prevents the migration of inflammatory cells from circulation to tissues by blocking the synthesis of chemokines, pro-inflammatory cytokines, interferon (IFN)-γ, granulocyte macrophage colony-stimulating factor, and tumor necrosis factor alpha (TNF-α) (8).

By inhibiting inducible nitric oxide synthase (iNOS), cortisol prevents endotoxin- induced venous insensitivity to norepinephrine. This increases mean arterial pressure and systemic vascular resistance, and improves mean arterial pressure responses to norepinephrine and phenylephrine. Additionally, the need for vasopressor therapy and duration of shock can be decreased (9).

Although the rationale for replacement therapy with hydrocortisone in catecholamine-dependent septic shock is growing stronger, a short course of high doses of corticosteroids should not be administered in severe sepsis, except in specific conditions as listed in Table 1. (10).

Table 1. Indications for use of short-course, high-dose corticosteroids in severe sepsis.

- Typhoid fever
- Pneumocystis carinii pneumonia in AIDS
- Bacterial meningitis in children

Despite short course-high dose therapy, long courses of low-dose corticosteroids reduce mortality at 28 days in intensive care units, while they do not alter the risk of gastroduodenal bleeding, superinfections or hyperglycemia. According to one study, hydrocortisone (or equivalent steroid) could be given to patients with septic shock immediately after they undergo an adrenocorticotropin hormone test, at a dose of 200-300 mg, and should be continued for 5-11 days, only when absolute or relative adrenal insufficiency is present (11).

In contradiction to the previously cited study, corticosteroids did not make an impact on 28-day all-cause mortality or on hospital mortality in severe sepsis and septic shock. This was shown with a meta-analysis by Annane et al. (12) of a subgroup of five trials studying five-day courses of low-dose corticosteroids. The evidence from eight trials did not support the use of short course of high dose of corticosteroids in severe sepsis or septic shock. However, the results in each of these studies were heterogeneous.

A meta-analysis by Minneci et al. (13) published the same year as Annane's meta- analysis evaluating five randomized clinical trials concluded that short courses of high-dose glucocorticoids decrease survival during sepsis, while 5- to 7-day course of physiologic hydrocortisone doses with subsequent tapering increases survival rate and shock reversal in patients with vasopressor-dependent septic shock.

A recent prospective, randomized, double-blind, single-center study in a postopera¬tive intensive care unit in Brazil, which involved 29 patients with systemic inflammatory response syndrome (SIRS), showed that dexamethasone when administered as a single dose of 0.2 mg/kg improved the effects of vasopressor drugs and respiratory functions, albeit it did not block the evolution of SIRS (14).

Luce (15) recommends in his editorial that physicians should wait for the ongoing clinical study carried out by Sprung et al., called CORTICUS, which involves 800 patients, to test the hypothesis of whether hydrocortisone would reduce 28-day mortality by 10% in patients with septic shock whose cortisol levels do not increase above 248 mmol/L in response to corticotropin stimulation.

Immunoglobulins:

Despite the use of highly potent antimicrobial drugs, owing to the inappropriate intrinsic defense mechanism responses, many infections are still difficult to treat. Either failed response or overwhelming response might be responsible for treatment failure, which justifies the endeavours in modulating host defenses to cope with the insult. This modulation can be by inhibiting or enhancing the immune response. Stimulation of anti-inflammatory response and inhibition of proinflammatory response are particularly useful in sepsis.

Immunoglobulins are humoral antibodies secreted by B cells. Administration of intravenous immunoglobulins (IVIG) has been shown to exhibit anti-inflammatory properties. Although they induce proinflammatory cytokines shortly after infusion, their major effect is a downregulation of the inflammatory response (16).

Use of IVIG is a novel therapeutic effort to modulate immune response in sepsis. IVIG might exert beneficial effects in sepsis by several mechanisms (Table 2). IVIG prophylaxis can undoubtedly reduce the occurrence of infections, especially pneumonias, in high-risk patients and is used for the treatment of streptococcal toxic shock syndrome (17).

The history of IVIG use in sepsis dates back to 1981 (18), but there are still controversies surrounding their use. In 2002, a Cochrane review found that polyclonal Ig reduced mortality significantly among adults (19). A later meta-analysis in 2004, stemming from the fact that Ig had been the second largest drug cost in the author's

Table 2. Mechanisms of IVIG effects in sepsis.

- Toxin inactivation
- Neutralization of bacterial endotoxins, exotoxins and Gram-negative outer membrane proteins
- Lipopolysaccharide (LPS) clearance
- Increased LPS uptake in liver and spleen
- Decrease in bacterial cell adherence, cell invasion, and organ invasion
- Stimulation of leukocyte and serum bactericidal activity
- Neutrophil phagocytosis of E. coli in patients with Gram-negative sepsis
- Enhancement of serum opsonic activity
- Neutralization of bacterial mitogenicity
- Enhancement of Kupffer cell phagocytic function during the late phase of sepsis and endotoxemia
- Interference with cytokine effects
- Prevention of excessive complement activation

hospital, concluded that polyclonal Igs should not be used because a subgroup of four high-quality trials failed to demonstrate a reduction in mortality (20).

Laupland et al. (21) in their meta-analysis of 14 randomized clinical trials revealed that there is significant reduction in mortality in critically ill adult patients with severe sepsis and septic shock treated with polyclonal IVIG, although the methodological quality of the studies were questionable.

Kreymann and colleagues (22) extended the previous meta-analysis by including randomized controlled trials in adults, children, and neonates. They reviewed a total of 27 studies and the data were summarized separately for adults or older children and neonates. The authors determined that the trend for adult patients was composed of two approaches: using IgM- and IgA-enriched IVIG preparations (IGAM) or preparations that contained only IVIG. Their conclusion based on the data was that the IGAM preparation reduced mortality in adults by 34% and in neonates by 50%, leading them to think that there is sufficient evidence to support the use of such preparations in severe sepsis or septic shock.

The multicenter "score-based IgG therapy of patients with sepsis" (SBITS) study by Werdan and colleagues (23) using IVIG (n = 321) compared with human serum albumin (n = 303) as placebo control did not show a reduction in the 28-day mortality rate (39.3% vs.

37.3%, respectively). This study outweighs all other trials of IVIG in power and quality and can be considered a landmark trial. Werdan et al. recommended that IVIG should not be used as adjuvant in sepsis and septic shock, at least in adult patients. As a result, reduction in mortality seems to be due to IGAM more than by IVIG alone. IVIG are widely used as prophylaxis against and as supplemental treatment of sepsis and septic shock, although they are not currently approved as medical indications for IVIG products. More data are necessary to ascertain whether this beneficial effect is linked with a reduction of infection-related morbidity and mortality.

Anticoagulants:

Sepsis is associated with severe alterations in the blood coagulation and fibrinolytic systems. Severe sepsis is triggered by diverse pathogens that lead to secretion of pro- and anti-inflammatory cytokines, activation and mobilization of leukocytes, activation of coagulation and inhibition of fibrinolysis. This results in disseminated intravascular coagulation characterized by simultaneous widespread microvascular thrombosis and profuse bleeding from various sites.

Microvascular thrombosis then leads to tissue hypoperfusion and multiorgan failure. There are a number of endogenous pathways preventing thrombus formation in humans, which include the fibrinolytic system, antithrombin (AT), tissue factor pathway inhibitor (TFPI), and the protein C-protein S-thrombomodulin pathway (24).

Intravascular fibrinolysis is mainly regulated by plasminogen activator inhibitor (PAI).

PAI is a glycoprotein that is a member of the serine protease inhibitor family. Its primary action is inhibition of plasminogen activators, activated protein C (APC) and thrombin, thus acting as a procoagulant. It is an acute phase protein (25).

Sustained increased levels of PAI are associated with worse outcomes in sepsis (26). There are genetic polymorphisms in the gene encoding PAI that contribute to the end result in sepsis and multi-trauma patients (27,28).

In light of the aforementioned knowledge about PAI, it could be stated that PAI deserves attention as a potential therapeutic target in sepsis. Polymorphism analysis to identify patients at increased risk and medical inhibition of PAI are possible options for future therapy.

A novel antithrombotic role for high molecular weight kininogen is as an inhibitor of PAI function (29).

The other inhibitor of the fibrinolytic system is the thrombin activatable fibrinolysis inhibitor (TAFI). TAFI is synthesized in the liver and behaves as an anti-inflammatory substance. It was shown to induce hepatic necrosis in septic mice (30).

In a clinical study by Zeerleder et al. (31), it was shown that septic patients have lower TAFI antigen levels than normal controls. TAFI levels are stated to vary between patients due to polymorphisms. The levels of TAFI in circulation are also found to change between Gram-negative and Gram-positive sepsis (32). Any solid conclusion for TAFI as a possible treatment target can not be derived from the recent literature. More research has to be done for this purpose.

Tissue factor pathway inhibitor (TFPI) has been the point of attraction in sepsis treatment for some time. TFPI anticoagulates blood by both direct inhibition of factor Xa and by the factor Xa-dependent inhibition of the tissue–factor VIIa complex. The vast majority of the endothelium is in the microcirculation; thus, the highest levels of TFPI are also found in the microvasculature, which gives this substance a pivotal role in the regulation of microvascular thrombosis.

A phase 3 international trial with 1987 patients assessing the therapeutic effect of TFPI concluded that the therapy did not confer any survival benefit (33). LaRosa et al. (34) further commented in their article that protective mechanisms, dose, and the levels necessary to achieve effects of TFPI have to be determined in new trials. They criticized heparin in the previously mentioned trial as a confounding factor and reported that patients with severe pneumonia seem to be the subpopulation that would benefit from the TFPI therapy.

The anticoagulant actions of antithrombin (AT) are well known and are related to its function as an endogenous serine protease inhibitor. AT is a hepatically synthesized plasma protein.

It is rapidly consumed in sepsis by clearance with other activated clotting factors (35). AT levels are further diminished by enzymatic cleavage by neutrophil elastase production and by diminished hepatic synthesis during sepsis. In animal models of sepsis, AT III reduced the mortality rate and limited or even prevented development of organ dysfunction (36-40).

Stemming from its well-known anticoagulant and anti-inflammatory actions, an international clinical trial was held on AT. Unfortunately, no overall benefit was found by administration of 30 000 IU of plasma-derived AT over 4 days in a trial involving 2314 patients. It was observed that a prespecified subgroup of patients who received no heparin appeared to have some modest benefit from AT (P < 0.05) (41).

Despite findings of the trial, a later article evaluating the efficacy and safety of the AT therapy pointed out that treatment with high-dose AT may increase survival time up to 90 days in patients with severe sepsis and high risk of death and that the absolute risk reduction in sepsis mortality was maintained despite an increase in bleeding (42).

Activated protein C (APC) pathway is the classic negative feedback loop of anticoagulation. Activation of this pathway occurs along the surface of capillary endothelial cells. This activation is catalyzed by thrombin (43). APC is a potent serine protease and APC activity is facilitated several-fold by reversible binding to another hepatically synthesized, vitamin K-dependent protein known as protein S.

In addition to inhibition of fibrin formation, APC also promotes fibrinolysis in vitro by inhibiting two important inhibitors of plasmin generation, namely PAI-1 and TAFI (44).

APC has direct anti-inflammatory effects in experimental studies that are independent of the antithrombotic actions (45).

In vivo, the anti-inflammatory effects of APC include inhibition of neutrophil adhesion, decreased TNF elaboration, and decreased drops in blood pressure. APC has multiple effects in tissue culture systems, including limitation in nuclear factor (NF)-κB-mediated proinflammatory activity, inhibition of inflammatory cytokine and chemokine generation, and upregulation of antiapoptotic genes of the Bcl-2 family of homologs. Microbial mediators like lipopolysaccharide (LPS) can overcome the inhibition of apoptosis within endothelial cells. APC protects endothelial cells from apoptosis in experimental systems (46).

In experimental studies and in human sepsis, circulating blood levels of protein C rapidly decline, with loss of this important coagulation inhibitor function (47). The extent to which these protein C activators are downregulated in severe sepsis appears to vary widely (48).

These findings mentioned above provide the therapeutic rationale for the administration of APC in severely septic patients. In considering therapy with protein C pathway components, protein C supplementation is an obvious possibility, especially because protein C levels are decreased, sometimes severely, in severe sepsis.

In a phase 3 clinical trial called PROWESS (Protein C Worldwide Evaluation in Severe Sepsis), recombinant human APC administered by continuous infusion at a dose of 24 μg/kg per hour for 4 days reduced the mortality rate from 30.8% in the placebo group (n = 840) to 24.7% in the recombinant human APC group (n = 850; P = 0.005). This indicates an absolute reduction in mortality rate of 6.1% and a relative risk reduction of 19.4% associated with treatment with APC (49).

It was not long before the results of this trial met with opposing views. One of the criticisms was about the serious bleeding side effect of the drug, which occurred in 5.3% of the patients with 0.4% mortality (50). The other criticism concerned the obscure mechanism by which APC affects survival improvement (51).

A second study sponsored by the same company that supported the PROWESS study was held soon after the PROWESS study, this time called ADDRESS (ADministration of DRotrecogin alfa [activated] in Early stage Severe Sepsis). This study was basically designed to overcome the country-dependent limitations in indications for use of APC (52).

A Cochrane review published in 2007 suggested that APC should not be used in sepsis with an APACHE II score of less than 25 in pediatric patients and warns policy-makers, clinicians and academics to be cautious when promoting the use of APC in patients with severe sepsis and an APACHE II score of 25 or greater (53).

In addition to the above-mentioned data, a large randomized, placebo-controlled trial with APC in pediatric sepsis has recently been terminated prematurely because of lack of efficacy (54). As a result, a randomized controlled trial should be done before any conclusions regarding its clinical use can be made.

Future Implications for Sepsis Therapy

Anticoagulants:

Although APC is the only agent that is currently Food and Drug Administration approved for treatment of sepsis, several other agents look promising based on the results of preclinical studies in animal models or observational studies in patients.

One such compound is the ester ethyl pyruvate. The biochemical basis for the effects of ethyl pyruvate in animal models of sepsis is not completely understood. It is known that ethyl pyruvate inhibits the activation of NF-kB in in-vitro and in-vivo systems by covalently modifying an essential sulfhydryl group in the protein (55).

A compound that is closely related to the tautomeric form of ethyl pyruvate, called 2-acetamidoacrylate, has been shown to have antiinflammatory properties both in vitro and in vivo (56).

Venkataraman et al. (58) evaluated the effects of ethyl pyruvate in a rat model of arterial hypotension induced by the intravenous injection of LPS. The rats were randomized to resuscitation with either ethyl pyruvate solution or Ringer's lactate solution. Resuscitation with ethyl pyruvate solution significantly prolonged survival time. Resuscitation with ethyl pyruvate solution was also associated with significantly lower circulating concentrations of nitrite or nitrate (markers of nitric oxide synthesis) and interleukin (IL)-6, and higher plasma levels of IL-10.

Ulloa et al. (59) investigated the effects of ethyl pyruvate on LPS-induced inflammatory responses in vitro and in vivo. In these studies, they found that ethyl pyruvate inhibited the release of TNF and decreased levels of TNF mRNA. Ethyl pyruvate blocked the activation of the NF-kB and downregulated activation of p38 mitogen activated protein kinase, which is another proinflammatory intracellular signaling protein. The effects of ethyl pyruvate in this study were significant both before and after LPS challenge.

Insulin Receptors:

Glycogen synthase kinase-3 (GSK-3) belongs to the family of serine/threonine kinases and phosphorylates glycogen synthase leading to its inactivation, reducing glycogen synthesis (59). Pharmacological inhibition or genetic deletion of GSK-3β downregulates expression of NF-kB-dependent genes (60).

It is known that tight glucose control via administration of insulin improves survival and decreases organ dysfunction in critically ill patients. Insulin uses GSK-3β in glucose regulation, but it is not clear whether insulin exerts its effects in the critically ill via GSK-3β (61). There are experimental sepsis models that utilize selective GSK-3β inhibitors proving beneficial effects (62).

Statins:

Statins are used in clinical practice for their lipid-lowering effects, reducing cardiovascular morbidity and mortality in patients with and without coronary disease. In addition, statins have anti-inflammatory properties, such as deactivating NF-kB and activator protein 1, which are mediated by blocking the synthesis of isoprenoid intermediates (63,64).

These intermediates serve as attachments for intracellular signaling molecules. Statins interfere with leukocyte endothelial cell interactions by downregulation of the expression of integrin molecules, including CD11a, CD18 and leukocyte function antigen 1 (65-67).

It is known that pre- or post-treatment with statins improves survival in a murine model of sepsis (68), and even when human volunteers were pretreated with simvastatin before being injected with a small dose of LPS, circulating levels of several proinflammatory markers were significantly decreased (69). Decreased expression of pathogen-associated molecular pattern recognition receptors and Toll-like receptor (TLR) 2 and TLR4 accompanied (70).

Almog et al. (71) reported in their prospective observational cohort study in patients with acute bacterial infections that former treatment with statins reduced the rate of severe

sepsis and intensive care unit admissions. Thomsen et al. (72) used a population-based surveillance cohort design and demonstrated a significantly lower risk of dying in statin users at 180 days after admission for a bacteremic episode. Despite these two reports, Fernandez et al. (73) showed higher hospital mortality rates in statin-treated patients admitted with bacteremia.

In Canada, Hackam et al. (74) carried out a population-based cohort analysis in which half of the patients received a statin and half did not. The incidence of sepsis was lower in patients receiving statins than in controls even when confounding factors were eliminated.

Toll-like Receptors (TLRs):

Innate immune recognition relies on a diverse set of germ line–encoded receptors, termed pattern recognition receptors (PRR), which recognize broad classes of molecular structures common to groups of C1. TLRs are the largest family of PRR (75). Cell surface TLRs recognize bacterial, fungal and protozoan pathogens by recognizing external molecules on these organisms. Ligand binding to TLR results in conformational changes leading to recruitment of adaptor proteins. Engagement of these adaptors activate a series of signal transduction molecules including IL-1R–associated kinases, TNF receptor associated factor 6, transforming growth factor β activated kinase, and the inhibitor of NF-κB kinase complex. These events lead ultimately to activation of many inflammatory pathways such as IFNs, TNF-α, IL-1, IL-6, IL-10, IL-12 and chemokines (76-78).

Septic shock is caused by TLR stimulation in the setting of bacterial infection, leading to studies focusing on TLR antagonists as potential therapies for this condition (79).

Two TLR4 antagonists (E5564 and TAK-242) have already been shown to be effective in the

prevention and treatment of LPS-induced shock in animal models (80,81). Both were found safe in phase 1 and 2 clinical trials. In one clinical trial, E5564 protected healthy adults from the physiological changes associated with LPS challenge (82).

Phase 3 clinical trials will determine the effectiveness of TLR4 antagonists in the treatment of patients with sepsis.

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