Severe sepsis, coagulation, and fibrinolysis: Dead end or one way?

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It was suggested more than 30 yrs ago that inhibition of the clotting cascade by natural anticoagulants could decrease the high mortality observed in patients suffering from severe sepsis and septic shock. Unfortunately, this therapeutic “paradigm” has led to a dead end, illustrated by the failure of all randomized trials and the recent withdrawal of recombinant activated protein C. Should we now definitely give up trying to treat septic coagulation disturbances or is there any therapeutic alternative? (Crit Care Med 2012; 40:2704–2708)

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In severe sepsis, dysregulation of the hemostatic system may lead to disseminated intravascular coagulation (DIC) and result in microvascular thrombosis, hypoperfusion and ultimately multiple organ failure, and death (1). Therefore, it was suggested >30 yrs ago that inhibition of the clotting cascade by natural anticoagulants could decrease the high mortality observed in patients suffering from severe sepsis and septic shock. At the same time, a considerable number of experimental and clinical studies demonstrated that most mediators of coagulation and fibrinolysis are implicated in the inflammatory network. They also showed that natural anticoagulants act as “anti-inflammatory” proteins, by modulating intracellular signaling, cytokine secretion, cellular or lymphocyte apoptosis, and leukocyte-endothelial interactions (2). These findings reinforced the justification for using these inhibitors as early as possible to treat severe sepsis. Unfortunately, this therapeutic “paradigm” has led to a dead end, illustrated by the failure of most randomized trials and the recent withdrawal of recombinant activated protein C. Should we now definitely give up trying to treat septic DIC or is there a therapeutic alternative?

Univocal Sequence of Coagulation and Fibrinolysis Activation

Following bacterial invasion, activation of hemostasis follows a univocal sequence. The process begins by expression of tissue factor on phospholipid membranes, followed by thrombin generation and fibrin formation. This procoagulant step is followed by an early fibrinolysis due to increased expression of plasminogen activators (t-PA and u-PA). Fibrinolysis is then rapidly inhibited by an increased synthesis of plasminogen activator inhibitor-1. In animal models, this characteristic sequence induces a procoagulant and antifibrinolytic state in <3 hrs (3). In humans, it could occur more progressively. Once septic injury is controlled, this hemostatic imbalance vanishes in 4–6 days, with a final progressive fibrinolytic stage (Fig. 1). Depending on the patient’s underlying situation and bacterial virulence, the hemostatic sequence may be “explosive” (inducing widespread thrombosis and hemorrhages in a few minutes) or “progressive” and often clinically imperceptible, although always detectable by specific biological tests. Thus, intensity and timing of secondary resolution may differ from patient to patient. The final restoration of the hemostatic balance depends on individual protein synthesis capacity and efficacy of natural coagulation inhibitors.

Natural Anticoagulants Are Determined to Limit Thrombin Generation

Three principal natural anticoagulants have antithrombin (AT) properties. The tissue factor pathway inhibitor inhibits the effect of the tissue factor VII complex and factor X on prothrombinase complex, thus suppressing the primary steps of thrombin generation (4).

AT is a direct 1/1 thrombin inhibitor, leading to thrombin–antithrombin complex formation and subsequent elimination. It binds with endothelial glycosaminoglycans, serving as cofactor. Heparin decreases AT endothelial affinity, which limits its anti-inflammatory effects (2, 5).

Protein C (PC) is a vitamin K–dependent protein, synthesized in the liver as a zymogen. It is converted to its activated form (APC) by proteolysis on the thrombin–thrombomodulin complex (IIa-TM). This activation is enhanced by the endothelial PC receptor. APC is a potent inhibitor of thrombin generation through irreversible inhibition of factors Va and VIIIa. APC also acts as a profibrinolytic mediator by inhibiting plasminogen activator inhibitor-1 and thrombin-activatable fibrinolysis inhibitor. In addition, APC exerts numerous anti-inflammatory effects (6).

These three inhibitors act together to limit excessive thrombin generation and induce a significant degree of anticoagulation, depending on the inhibitor but also on its concentration, half-life, activity of cofactors, and associated hemostatic disturbances. Unsurprisingly, a significant number of hemorrhagic adverse effects were observed in the randomized trials testing these three inhibitors. Post hoc analysis of the optimized phase 3 tifacogin in multicenter international sepsis trial r-tissue factor pathway inhibitor and KyberSept AT trials was consistent with a causal relationship between hemorrhagic events and association with heparin. In the Recombinant human protein C Worldwide Evaluation in Severe Sepsis (PROWESS) APC trial, the frequency of bleeding events was multiplied by two (7–9).
Activation of Hemostasis Represents a Potent Mechanism of Innate Defense Against Bacterial Invasion and Dissemination

The purpose of activation of hemostasis is generation of blood clots following traumatic injury. It is also a potent innate antibacterial mechanism. Following bacterial invasion, formation of a fibrin network allows microbial trapping, and thus limits bacterial growth through decreased availability of oxygen and nutrients. It facilitates leukocyte engagement and blocks dissemination. Conversely, reducing thrombin generation increases susceptibility to infection, suggesting that plasma fibrinogen might be an important host factor against microbial infection. These findings may also explain the evolution of plasma fibrinogen level as an acute phase reactant (10–14). The interplay between innate immunity and blood coagulation is reinforced by the recent description of the antibacterial and prothrombotic effects of neutrophil extracellular traps. These extracellular chromatin threads, comprised of histones and granular proteins released from the neutrophil nucleus after leukocyte activation, facilitate bacterial entrapment and killing. They also enhance platelet adhesion and aggregation, increase proteolysis of natural anticoagulants, impair TM-dependent PC activation, and thus activate the coagulation process (15).

To circumvent these mechanisms of defense, bacteria have developed specific fibrinolytic properties. Infections induced by Yersinia species have been particularly studied as models of this microbial “corruption” of hemostasis (16). To destroy the fibrin network, bacteria express specific proteases acting as plasminogen activators and use host plasminogen to generate fibrin proteolysis. Thus, bacterial plasminogen/plasmin-mediated clearance of fibrin provides a means of escape from fibrin immobilization and supports microbial evasion of inflammatory cells. Almost all bacteria possess fibrinolytic properties. Overall, these findings represent a strong argument for a beneficial effect of coagulation activation and fibrinolysis inhibition occurring after microbial invasion. Experimental studies support this hypothesis. For instance, in the murine model of pneumonia induced by Pseudomonas aeruginosa, an early

Figure 1. Sequence of coagulation and fibrinolysis (adapted from reference (3) and (25)). A, sequential changes in tissue-plasminogen activator (t-PA) and plasminogen-activator inhibitor (PAI-1) plasma levels after experimental endotoxin infusion in piglets. Note the initial increase in t-PA rapidly inhibited by PAI-1. B, sequential changes in PAI-1 and plasmin-antiplasmin complexes (PAP) plasma levels in human septic shock. Note the progressive decrease in PAI-1 levels mirror of increased PAP levels. C, sequential changes in antithrombin (AT) and protein C (PC) activity in human septic shock. Note the decreased AT and PC levels occurring at day 1 and progressive normalization at day 5.
inhibition of thrombin by recombinant AT of APC did not modify inflammation, increased alveolar endothelial permeability, and exacerbated lung pathologic changes (17–19). Overall, the potential risk induced by thrombin inhibition at the early phase of sepsis should be kept in mind.

Sepsis-Induced Activation of Hemostasis Is Modulated by Acute Changes in Synthesis and Regulation of Inflammation Proteins

Procoagulant changes in sepsis are usually considered to be pathological, leading to so called “consumption coagulopathy,” where coagulation factor levels are decreased through step-by-step proteolysis. The decrease in coagulation factors is used as proportional marker of coagulation activation for the diagnosis of DIC. The same mechanism of consumption is thought to explain the decrease in the plasma levels of natural antiocoagulants.

In fact, this interpretation may be false. In the sepsis-induced DIC process, some mediators are upregulated and some downregulated. Fibrinogen and factor V are positive acute phase proteins, while hepatic synthesis of the natural anticoagulants AT and PC is rapidly and significantly decreased. These inhibitors should be considered negative acute phase proteins (20, 21). This explains why decreased AT or PC activities are observed in septic patients even though their coagulation appears moderately activated (22). Sepsis-induced coagulopathy is the overall result of proteolytic consumption in the fibrin generation process and of complex inflammation-induced changes in protein synthesis. Thus, decreased activity of thrombin inhibitors should be interpreted as participating in the host defense mechanism.

Excessive Activation of Coagulation and Major Decrease in Anticoagulant Levels Are Strongly Correlated With Death and Organ Failure

Contrasting with the determinism of coagulation activation as a host defense mechanism, excessive deregulation of hemostasis is associated with subsequent organ failure and death. A large number of clinical studies have confirmed this finding. As a whole, a high DIC score is strongly associated with mortality and in some studies stronger than general severity scores. Concerning fibrinolysis, the correlation between the secondary increase in plasminogen activator inhibitor-1 levels and organ failure is supported by numerous studies. In patients suffering from septic shock, a lack of correlation between thrombin–antithrombin and plasmin–antiplasmin (PAP) complexes, revealing a sustained coagulation activation without secondary fibrinolysis, was highly predictive of poor outcome (23). These findings support the hypothesis that prolonged and disproportionate coagulation and antifibrinolysis are at least partly responsible for organ failure. Indeed, some promising results were reported when treatment with recombinant tissue-plasminogen activator was used to restore fibrinolysis (24). The high risk of hemorrhagic adverse effects precluded the possibility of carrying out further studies.

Similarly, sequential studies of the natural coagulation inhibitors AT and PC were equally consistent with a correlation between severely decreased plasma levels and death or organ failure (25). Numerous observational studies showed an initial decreased AT or PC activity to be highly predictive of poor outcome. Interestingly, in two studies, a secondary decrease in AT and PC activities within the first 24 hrs predicted death and organ failure more accurately than baseline measurements (26, 27).

Misconceptions in the Randomized Trials of Natural and Activated Anticoagulants

Unfortunately, the possibility that coagulation activation and inhibition of fibrinolysis could participate in host defense against microbial dissemination was not considered when therapeutic trials were designed. It was universally accepted that inhibition of coagulation should be begun as early as possible and with sufficiently high doses of inhibitors to block fibrin formation and modulate inflammation. Anti-inflammatory properties of natural inhibitors were considered to be of major benefit. High doses were justified by the hope of eliciting these properties. They were supported by animal studies showing that only “supranormal” plasma levels were able to modulate inflammation, leukocyte adhesion, microcirculatory changes, and apoptosis (28).

In addition, this was another justification for using early high doses; the three inhibitors were considered dysfunctional. More than an adaptive sepsis-induced downregulation of synthesis, the early and acute decrease in plasma levels of AT was explained by consumption, protease-induced inhibition, and extravascular leakage due to increased endothelial permeability (2, 5). Concerning the PC system, downregulation of TM expression was considered the main mechanism explaining the decreased APC synthesis (29).

With this paradigm, the same size was thought to fit all: irrespective of the time period of sepsis, of the bacteria, of the degree of coagulation activation, all patients were given the same inhibitor, at the same high dosage, and for the same time. Therapeutic use of PC zymogen was excluded because its endothelial activation was considered dysfunctional, insufficient or unpredictable, contrasting with clinical findings showing that a significant degree of PC activation remained and could induce significant APC levels (30). The possibility that hemorrhagic adverse effects could be lowered by AT concentrates alone or PC concentrates instead of APC was not considered. Association of AT with heparin was permitted to facilitate inclusions by physicians who routinely used preventive or curative anticoagulation. Above all, randomized trials of tissue factor pathway inhibitor, AT or APC, were designed to include patients without any criteria of coagulation activation and irrespective of its time sequence. Illustrating this fact, the recently published Captivatate recombinant tissue factor pathway inhibitor trial failed to document a reduction of mortality in severe community-acquired pneumonia. In this trial, the proportion of patients suffering from DIC reached 4.8% only (31). Except for the pivotal PROWESS study, all these trials failed, but their results bring additional strong support to the possibility that coagulation inhibitors remain a useful treatment. In all studies where it was documented, post hoc analysis showed that overt coagulation activation was strongly associated with mortality or organ failure and also with the best therapeutic effect of the inhibitor (32–36) (Table 1).

Later analysis of the PROWESS and further APC trials consistently showed a relation between the effect of the drug and its early administration. However, in the more severely ill patients who
benefited from the treatment, criteria for overt DIC were already fulfilled at inclusion. On the other hand, no effect or even a trend to an increased mortality could be documented in the less severe patients who did not exhibit excessive coagulation activation. This is in agreement with a possible adverse effect of APC when used “preventively” contrasting with its favorable effect on DIC. In addition, a significant reduction of mortality was observed in open trials of AT or recombinant TM, when overt DIC was required for inclusion (37, 38). Unfortunately, the Atryn trial designed to document the effect of recombinant AT in septic shock with DIC could not be completed. However, phase 2 and phase 3 trials of a new recombinant AT and of recombinant TM are underway in Japan and may bring additional data supporting this hypothesis.

Since secondary restoration of fibrinolysis by plasminogen activators carries a high risk of bleeding, treatment of DIC should be primarily directed against overt coagulation. Until their efficacy on inflammation is confirmed, “supranormal” doses and activated anticoagulants (APC, AT-heparin association) should not be used to limit the risk of bleeding. Therapies should use replacement of natural anticoagulants at doses calculated daily to rebalance excessive coagulation activation. From my point of view, there is still a place for a specific treatment of septic DIC.

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