**Success or Failure in Phase III Sepsis Trials: Comparisons between the Drotrecogin Alfa (Activated) and Antithrombin III Clinical Trials**

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The success of the Phase III Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) trial of drotrecogin alfa (activated) in the treatment of severe sepsis provides an opportunity to contrast this experience with similar, yet unsuccessful, sepsis trials. The KyberSept multicenter trial of antithrombin III for severe sepsis was a contemporaneous study, which was compared with the PROWESS trial. An analysis of trial designs, population differences, and study endpoints may affect how future clinical trials are developed and conducted, and may account, in part, for the success of the PROWESS trial and the failure of the KyberSept trial to reach the primary endpoint of reduced mortality in severe sepsis. This review is an examination of both the final clinical trial reports from the KyberSept and PROWESS trials, provided in published abstracts, papers, and meeting reports, and supporting preclinical and clinical evidence published over the past 20 years. While both these highly visible clinical trials used endogenous anticoagulants that down-regulate the generation of thrombin in vivo, important differences exist in their anti-inflammatory properties, safety profiles, and antithrombotic mechanisms.

The successful conclusion of the Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) trial of drotrecogin alfa (activated) — the official generic nomenclature for recombinant human Activated Protein C — in severe sepsis represents a long awaited milestone in clinical sepsis research [1]. This multinational Phase III trial successfully achieved a statistically significant reduction in 28-day all-cause mortality in an intention-to-treat analysis, when comparing the drotrecogin alfa (activated) group with the placebo control group. Eligible patients received either drotrecogin alfa (activated) administered by continuous infusion at a dose of 24 µg/kg/h for 4 days, or placebo. The mortality rate in those patients randomized to the drotrecogin alfa (activated) group (n=850) was 24.7%, while patients in the control group (n=840) had a mortality rate of 30.8% (p=0.005). This indicates an absolute reduction in mortality rate of 6.1% and a relative risk reduction (RRR) of 19.4% associated with drotrecogin alfa (activated) treatment. These results are a welcome departure from the almost uniformly disappointing outcomes from a myriad of other recent, randomized clinical trials for severe sepsis.

Over the past 15 years, more than 20 similarly designed, multicenter sepsis trials with other agents have failed to demonstrate a statistically significant reduction in overall mortality [2,3]. It is worth noting that in three such clinical trials (tumor necrosis factor (p75):Fc fusion peptide [4], nitric oxide synthase inhibitor [5], and growth hormone [6]), patients randomized to the treatment groups actually experienced significantly higher mortality rates (p<0.01) than the placebo control groups. The highly favorable results observed in the PROWESS trial have not been seen in sepsis trials since the original Ziegler study with *Escherichia coli* J5 antisera for the treatment of Gram-negative bacteremia, almost 20 years ago [7]. Despite major advances in the understanding of the molecular pathogenesis of sepsis, it
has been frustratingly evident that improvements in the management of patients with septic shock had not been forthcoming [3]. The pessimism that has pervaded the field of clinical sepsis research has been broken by the remarkably successful results of the PROWESS trial.

While the PROWESS trial was being conceived and initiated, another Phase III sepsis trial with a comparable endogenous human anticoagulant, antithrombin (formally known as antithrombin III), was underway. This KyberSept trial was an international, multi-center, placebo-controlled, double-blind, randomized clinical trial involving over 2300 patients [8]. Antithrombin, or placebo, was administered by an initial bolus of 6000 IU followed by continuous intravenous infusion of 6000 IU for 4 consecutive days. In contrast to the favorable results achieved in the PROWESS trial, the KyberSept trial did not meet its primary goal of a significant reduction in 28-day all-cause mortality. The overall mortality rate, 28 days after trial entry, was 38.9% in the antithrombin group (n=1157) and 38.7% (p=NS) in the placebo group (n=1157). These results were particularly disappointing in that treatment with antithrombin had been convincingly demonstrated to be of survival advantage in multiple preclinical sepsis models [9], several Phase II trials [10,11], and numerous small non-randomized clinical studies [12,13].

Why is it that two similarly designed clinical trials, with agents with seemingly comparable mechanisms of action, run contemporaneously in similar patient populations, resulted in such disparate results? Prior to the initiation of these trials in the late 1990s it would have been very difficult to predict which agent would ultimately prove to be successful in Phase III clinical testing. Are there important differences in the molecular actions of drotrecogin alfa (activated) and antithrombin as anticoagulants that account for the trial results? Can the demonstrable anti-inflammatory properties of drotrecogin alfa (activated) and antithrombin explain the differences in the Phase III trial results? Were there substantive variations in the conduct and design of the clinical trials that led to success of the PROWESS trial and failure of the KyberSept trial? Now that these studies are complete, and initial trial reports are available, it is possible to critically analyze these studies in an effort to understand the disparities in trial outcome.

Mechanisms of action
Are the mechanisms of action on the coagulation system of Activated Protein C (herein referring to the native molecule) and antithrombin sufficiently different to explain the Phase III trial results? The Activated Protein C pathway and antithrombin are both major endogenous inhibitors of the human coagulation system. The central importance of coagulation and fibrinolytic systems in the pathogenesis of microcirculatory failure and organ dysfunction in sepsis is now well recognized [14–16]. It is also increasingly appreciated that the coagulation networks and the innate immune system are highly integrated and co-regulated. Modulation of pro-thrombotic activity also attenuates the pro-inflammatory cytokine networks, and visa versa [16,17]. It has been assumed, for a decade or more, that control of the innate immune response in sepsis would be able to prevent the acquired coagulopathy of sepsis [18]. Early clinical trials with heparin and other anticoagulants as a treatment strategy for the disseminated intravascular coagulation (DIC) that often accompanies sepsis were not consistently effective in improving the survival of patients with severe sepsis [19,20]. The coagulation abnormalities observed in sepsis were largely viewed as a late event in the pathophysiology of septic shock, and were relegated to a secondary epiphenomenon [18]. It has become increasingly evident that thrombin generation is a very early event following systemic injury and infection, and that coagulation plays a critical role in the underlying microcirculatory events that culminate in septic shock [14–17,21].

Both antithrombin and Activated Protein C function as major inhibitors of thrombin generation and fibrin deposition following coagulation activation. Both of these endogenous anticoagulants are consumed in the presence of septic stimuli and their levels fall commensurate with the magnitude of systemic immune and clotting activation [14–16]. Low circulating levels of both these proteins are correlated with high mortality rates in septic shock [21–24]. This loss of regulation of the coagulation and fibrinolytic systems is well recognized as a good prognostic indicator of poor outcome in the severely septic patient [25], and this forms the therapeutic rationale for replacement therapy with either antithrombin or drotrecogin alfa (activated) in clinical trials for severe sepsis.

Despite similar physiological roles in the regulation of clotting, the molecular mechanisms by which they achieve an anticoagulant state are fundamentally different. The unique structural and physiological differences between Activated Protein C and antithrombin may explain, in part, the disparate results of the two recently completed Phase III clinical trials.

Mechanisms of action of Activated Protein C
The Activated Protein C pathway of anticoagulation is a classic negative-feedback loop, induced by thrombin generation itself. Activated Protein C circulates as
Protein C, an inactive precursor molecule (zymogen) that is transformed into the physiologically active enzyme by the proteolytic action of thrombin. This interaction only occurs when thrombin is complexed with its specific membrane-bound protein receptor, thrombomodulin, found abundantly on the luminal surface of capillary endothelial cells. Once bound to thrombomodulin, thrombin can no longer act upon fibrinogen as a substrate for conversion to fibrin and, therefore, ceases its pro-coagulant actions [26,27]. Nonetheless, the thrombin–thrombomodulin complex is capable of substrate binding to Protein C. Thrombin now becomes an anticoagulant enzyme as it converts Protein C to Activated Protein C. The thrombin-mediated cleavage of 12 amino acids from the amino terminus of the heavy chain of Protein C generates the anticoagulant enzyme Activated Protein C, a highly reactive serine protease with a short plasma half-life of approximately 15 min [22,24]. Down-regulation of thrombin formation by Activated Protein C is accomplished through its direct proteolytic degradation of coagulation factors V and VIII in the common and intrinsic clotting pathways, thereby inhibiting the down-stream generation of thrombin.

Activated Protein C is a relatively ineffective enzyme on its own, and Activated Protein C must link with another hepatically synthesized, vitamin K-dependent protein, known as protein S, to accomplish its full complement of proteolytic functions. This ‘accessory’ protein associates with Activated Protein C only in its free circulating form; protein S bound to C4b binding-protein from the complement system cannot bind to Activated Protein C. Since C4b binding-protein is an acute phase reactant and is elevated in inflammatory states such as sepsis, it is likely that, in sepsis, free protein S is not available in adequate amounts to promote Activated Protein C activity.

In addition to inhibition of fibrin synthesis, Activated Protein C also promotes fibrinolysis in vitro by the inhibition of two important inhibitors of plasmin generation: plasminogen activator inhibitor-1 and thrombin activatable fibrinolysis inhibitor. This pro-fibrinolytic activity of Activated Protein C is not shared by antithrombin [14–16,22]. Whether this difference accounts for the different trial results remains to be demonstrated. Inhibition of clotting by either Activated Protein C or antithrombin is expected to have demonstrable anti-inflammatory activity as a result of thrombin inhibition. Intravascular thrombin generation is highly inflammatory within the microcirculation via interaction with specific receptors on platelets, endothelial cells, and white blood cells known as protease activated receptors (PARs) [28]. Activation of these cells by PARs leads to increased cytokine and chemokine synthesis [29], platelet activating factor and histamine synthesis [30], and up-regulation of P-selectin expression [31]. Thrombin-initiated endothelial interleukin-6 (IL-6) production stimulates tissue factor expression and further perpetuates ongoing coagulation [32]. This positive feedback loop between clotting and inflammation terminates in DIC and septic shock (the ‘vicious cycle’ of clotting and inflammation of sepsis). All these actions promote neutrophil, lymphocyte, and platelet interactions with the capillary endothelium and this results in diffuse endothelial injury, increased vascular permeability, and cellular apoptosis. Inhibitors of intravascular coagulation, either antithrombin or Activated Protein C, would be expected to limit thrombin-mediated inflammatory states.

Recently, it has become evident that both antithrombin and Activated Protein C have direct anti-inflammatory effects that are independent of the antithrombotic actions of either protein. Activated Protein C is taken up by endothelial cells and white blood cells as a complex with a specific cellular receptor, known as endothelial Protein C receptor. This complex translocates to the nucleus and has multiple effects in tissue culture systems including [33]:

- limitation of nuclear factor-κB-mediated pro-inflammatory activity
- attenuation of inflammatory cytokine and chemokine generation
- up-regulation of anti-apoptotic genes of the Bcl-2 family of homologues.

Endothelial cells are relatively resistant to apoptosis as a result of the constitutive synthesis of a number of anti-apoptotic proteins [34]. Microbial mediators, such as bacterial lipopolysaccharide (LPS), can overcome the inhibition of apoptosis within endothelial cells. Activated Protein C protects endothelial cells from apoptosis in experimental systems. It remains to be demonstrated if this activity is relevant to the protective effects of Activated Protein C in human sepsis. A summary of the mechanisms of action of Activated Protein C is found in Figure 1.

**Mechanisms of action of antithrombin**

The anticoagulant actions of antithrombin are well known and relate to its ability to function as a potent endogenous serine protease inhibitor. Antithrombin is a hepatically synthesized serum protein that is activated by the process of allosteric activation by heparin, heparan, dermatan sulfate, and related glycosaminoglycans. Specific polysulfated pentasaccharides, found in repeating units in glycosaminoglycans and
mucopolysaccharides, are necessary to bind to a highly basic, central domain in antithrombin. A conformational change takes place in antithrombin following interactions with these acidic pentasaccharide moieties, bringing a critical arginine residue at position 393 to covalently link within the active site of serine proteases, thereby inactivating these proteases [13,35].

Many of the clotting factors and regulators of the coagulation system are serine proteases, including thrombin itself, factor Xa, several components of the contact system of coagulation, and tissue factor:factor VIIa–heparin complexes [36–38]. This broad-spectrum enzymatic action of antithrombin makes it a central inhibitor of the coagulation system. Antithrombin is consumed in the process of coagulation inhibition, neutrophil elastase production, and by diminished hepatic synthesis during sepsis. This loss of inhibition by reduced antithrombin levels contributes to the pro-thrombotic state that characterizes septic shock [13]. The role of antithrombin in coagulation inhibition and its anti-inflammatory actions in the microcirculation are depicted in Figure 2.

In the absence of therapeutic doses of heparin, antithrombin binds primarily to specific glycosaminoglycans on the cell surface of endothelial cells, such as heparan sulfate. In this configuration, antithrombin not only exerts local anticoagulant properties to capillary endothelium, but also promotes local anti-inflammatory activity [13,37]. This is mediated by antithrombin-mediated induction of prostacyclin synthesis by endothelial cells. Prostacyclin is a potent antiplatelet agent that:

- inhibits platelet aggregation and attachment
- inhibits neutrophil-endothelial cell attachment
- attenuates IL-6, IL-8, and tumor necrosis factor release by endothelial cells (Figure 2).

Antithrombin may also induce anti-inflammatory effects via signal transduction cascade coupled membrane proteins/receptors, such as syndecan-4 [39–41]. Antithrombin reduces expression of IL-6 and tissue factor and their corresponding intracellular mRNA levels. This correlates with the finding of the inhibition of activation of the transcription factor, nuclear factor-6B, in LPS-stimulated monocytes and endothelial cells [40,42,43]. Antithrombin significantly diminishes chemotaxis of neutrophils, monocytes, and lymphocytes by heterologous deactivation in IL-8 concentration gradients in Boyden chamber experiments. These activities may explain the attenuation of white cell–endothelial cell interactions in animal models.
employing intravital microscopy [44]. The administration of antithrombin to LPS-challenged animals significantly reduced the interaction of inflammatory cells with the vessel wall (characterized by rolling, sticking, and transmigration events), thereby limiting capillary leakage and subsequent organ damage [45]. These anti-inflammatory actions have been demonstrated in a number of experimental systems [39–53], and are presumably physiologically important within the microcirculation in human sepsis as well [54].

The mechanisms by which Activated Protein C and antithrombin inhibit coagulation and induce local anti-inflammatory actions in the tissues are similar, but not identical. Both Activated Protein C and antithrombin levels fall rapidly in sepsis and contribute to the ongoing pro-coagulant state that typifies severe sepsis. Is it possible that the differences in molecular mechanisms of anticoagulation and/or anti-inflammatory actions explain the success of drotrecogin alfa (activated) and failure of antithrombin in Phase III clinical testing? If this is true, it will require considerably more basic research to confirm the clinical relevance of these rather subtle mechanistic differences. Other potential explanations that follow in this paper seem to be a more likely explanation for the differences in clinical trial results between these two endogenous anticoagulants.

**Drug interactions with heparin and the risk of bleeding**

The risk of hemorrhage was greater in patients treated with drotrecogin alfa (activated) or antithrombin than in patients randomized to the placebo control group in both clinical trials. The magnitude of bleeding risk was greatest in patients who received antithrombin, yet even the placebo group in the antithrombin group appeared to have a significantly greater risk of hemorrhage. There are critically important questions to be answered in an effort to understand these differences in results:

- What accounts for this seemingly excess risk of hemorrhage in the KyberSept trial?
- Were patients enrolled in the antithrombin trial more unwell, or at greater intrinsic risk of bleeding, independent of the treatment administered?
- Is antithrombin a more potent coagulation inhibitor than Activated Protein C?
- Was the dose of antithrombin too high in this Phase III trial?
- Did allowances for concomitant heparin administration, or other anticoagulants, during the conduct of the trial, affect the overall bleeding risk?
- Did differences in the trial designs and the definitions of hemorrhagic events account for the excess reports of bleeding in the KyberSept trial?
It is the authors’ contention that a combination of events explains the significantly different bleeding risk observed in these clinical trials.

The study populations may have differed in these studies, since the overall 28-day all-cause mortality rate in the placebo groups were 38.7% in the KyberSept trial and only 30.8% in the PROWESS trial (p=0.01). These differences in overall mortality rates are probably related to the entry criteria used in these studies that created a more severely ill patient population in the KyberSept trial. The degree to which this difference contributed to the differential risk of bleeding in these studies is speculative at this point, and difficult to accurately quantify. Nonetheless, a few elements clearly differ between these studies, and may account, at least in part, for the observed variation in bleeding risk.

The exclusionary criteria used to limit the risk of hemorrhage appear to be remarkably similar between the two study groups [1,8], but the definitions of severe hemorrhage differ substantially. The overall risk of severe hemorrhage was 5.7% in the placebo group in the KyberSept trial while the comparable number in the PROWESS trial was 2.0% (p<0.0001). Much of this apparent difference is related to the definition of major hemorrhage used in the two studies. Major hemorrhage in the KyberSept trial was defined as any intracranial bleeding and/or any bleeding that required at least 3 units of transfused packed red blood cells (RBCs) within 24 h, throughout the 28-day observation period. In the PROWESS trial, significant bleeding was defined as any intracranial bleeding and/or life-threatening bleeding (risk of death at time of event), and/or any bleeding that required ≥3 units of RBCs on each of two consecutive days (within the 4-day treatment period +8 h). The apparently lower bleeding risk may have been a reflection of the more strict criteria (bleeding for 2 consecutive days) used to define major hemorrhage in the PROWESS trial. A bleeding event that required 4 units of packed RBCs over 24 h was classified as severe hemorrhage in the KyberSept trial, but not in the PROWESS trial.

The likelihood of recent surgery in the patient population was 40% in the KyberSept trial, while it was approximately 27% in the PROWESS trial [1,8]. This may also account for some of the excess bleeding events observed in the KyberSept trial.

One of the most remarkable differences between the two trials, with respect to bleeding risk, remains the treatment interaction with heparin. Heparin was allowed in both studies at doses that would not be considered adequate for systemic anticoagulation. The KyberSept trial allowed up to 10 000 IU of heparin/day during the study-drug infusion, while the PROWESS trial allowed up to 15 000 IU of heparin/day. Remarkably, the overall risk of hemorrhage was not different in heparin-treated study patients given concomitant drotrecogin alfa (activated) compared with the placebo group (3.5% vs. 3.7%; p=NS) [1]. This was certainly not the case with antithrombin therapy [8]. In contrast to patients who did not receive heparin, the risk of major bleeding was statistically significantly greater in patients receiving concomitant heparin (10.9% with antithrombin vs. 6.2% in the control group; p<0.01).

This treatment interaction with heparin and antithrombin was not entirely unexpected, since heparin is known to potentiate the anticoagulant activity of antithrombin [9,12,13]. It was hypothesized that limiting heparin to essentially prophylactic doses only would not be associated with an excess bleeding risk. This hypothesis appears to be in error. At the high doses of antithrombin used in this trial, a treatment interaction with even low doses of heparin increased the bleeding risk in severely septic patients. Moreover, heparin interferes with the ability of antithrombin to bind to heparan sulfate on capillary endothelial surfaces. Loss of binding to endothelial membranes may result in the reduction of local anticoagulant activity in the microcirculation, but also blunts antithrombin-induced prostacyclin synthesis by endothelial cells. Antithrombin’s abilities to limit inflammatory mediator release, and its modulating effect on neutrophil–endothelial cell interactions along the vessel wall, are predicated upon antithrombin binding to endothelial surfaces [40,41]. In this manner, heparin also removes the local anti-inflammatory properties of antithrombin [43–45,55].

A careful review of the KyberSept trial data reveals that concomitant heparin administration greatly affected the efficacy of antithrombin. If the predefined subgroup of patients who received antithrombin without heparin is compared with non-heparinized placebo patients, the 90-day overall mortality rate is significantly reduced (p<0.05) with an approximate 15% RRR over the placebo patients. This subgroup accounted for about 700 patients (30%) of the entire study population. If those patients who did receive heparin during the study treatment phase are analyzed, antithrombin had no demonstrable benefit. This may have been a consequence of both a loss of efficacy and excess bleeding risk in the antithrombin plus heparin subgroup [8].

**Trial design, entry criteria, and baseline characteristics**

Could other small differences in trial design, entry criteria, or baseline characteristics have contributed to the
failure of the KyberSept trial, while the PROWESS trial was a success? The enrollment processes in both trials were very similar. Patients were enrolled if they met clinical criteria within a preset window of eligibility and gave informed consent. In both studies, entry criteria included clinical evidence of sepsis, signs consistent with a systemic inflammatory response syndrome (SIRS), and evidence of organ dysfunction.

In the PROWESS trial, criteria for enrollment included evidence of known or suspected infection and three of the following:

- hypo- or hyperthermia
- tachycardia
- tachypnea
- leukocytosis or leukopenia.

In addition, there had to be dysfunction of at least one organ system. If the criteria were present in a 24-h period and the organ dysfunction was not >24-h duration, the patient was eligible. This was an attempt to enroll patients with potentially reversible physiologic derangement. Patients with long-standing, established organ dysfunction were excluded by this entry requirement.

In the KyberSept trial, a patient had to have severe sepsis as evidenced by a suspected source of infection, hypo- or hyperthermia, leukocytosis, or leukopenia, and three of the following:

- tachycardia
- tachypnea or mechanical ventilation
- hypotension despite appropriate volume resuscitation
- thrombocytopenia
- primary metabolic acidosis
- persistent oliguria despite sufficient fluid replacement.

All of these had to be present within a 6-h period. Every effort was made to enroll patients in the early phase of severe sepsis in order to select the high-risk patient population, in which a favorable effect was expected based on Phase II data. Yet this was not always the case. A patient was still eligible if five of the six criteria were present for many hours, or even days, before the sixth criterion was met. This may have resulted in the enrollment of patients who may be in a later phase of sepsis with established organ dysfunction. This seemingly minor difference in trial design between the two trials could have reduced the likelihood of success with antithrombin in the Phase III results.

Review of baseline characteristics in the KyberSept trial revealed that slightly >50% of patients had baseline antithrombin levels that were <60% of normal functional levels (55% placebo group, 52% antithrombin group). The resting plasma concentration of antithrombin has been shown to be approximately 110–140 mg/L, with a serum half-life of about 48 h [46]. In septic shock, the serum half-life may be reduced to <18 h. As previously mentioned, the cause of decreased antithrombin activity is multifactorial:

- decreased hepatic antithrombin synthesis in sepsis [47], even in patients with normal liver function
- acute consumption evidenced by an increase in thrombin–antithrombin complexes [48]
- inactivation by elastase released from activated neutrophils [49].

Each of these factors contributes to decreased serum half-life, and results in the reduced antithrombin activity. Rapid depletion of antithrombin has been shown to be predictive of a fatal outcome and replacement improves outcome of experimental sepsis in animal models [13].

The PROWESS and KyberSept trials differed with respect to the duration of the window of eligibility for enrollment. In the KyberSept trial, the window of eligibility for enrollment was 6 h, while, in the PROWESS trial, it was 24 h. Would a window of enrollment eligibility of longer duration have allowed more patients to reach more reduced baseline levels of antithrombin activity? This is difficult to answer but, since antithrombin levels fall rapidly in severe sepsis, this seems an unlikely explanation for the unfavorable results in the KyberSept trial.

**Dosing considerations**

Could differences in the method of dosing explain the Phase III trial results? In the KyberSept trial, dosing of antithrombin was standard: patients randomized to the antithrombin group received a total of 30 000 IU of antithrombin administered as a loading dose of 6000 IU given over 30 min, followed by a continuous infusion of 6000 IU/day for 4 days. In the PROWESS trial, drotrecogin alfa (activated) was dosed by weight. Patients randomized to the drotrecogin alfa (activated) group received a dose of 24 µg/kg/h for 96 h.

Anthropometric differences between patients were not considered in the KyberSept trial. For a 70-kg adult, the dose of antithrombin was approximately 85 IU/kg given as a loading, and as a daily continuous infusion for 4 days. The cumulative dose by weight was about 429 IU/kg. The weight-adjusted dose would have been lower for patients weighing >70 kg.
While the KyberSept trial did not demonstrate improved outcome with antithrombin replacement therapy, studies in a variety of animal models did have positive results [9,56]. In pig, rat, and baboon, septic endotoxic models, DIC and death were prevented by pretreatment with high-dose antithrombin (≥250 IU/kg) [57–59]. Studies of induced sepsis in rats and guinea pigs showed that with higher post-treatment doses (≥500 IU/kg), mortality was reduced [60]. In addition, heparin was not used in these models.

Further evidence that the dose of antithrombin may have been too low is the lower than expected antithrombin levels in the blood after 24 h of antithrombin replacement. The patients randomized to the antithrombin group demonstrated an average 116% increase in antithrombin levels, to approximately 180% of normal circulating blood levels. It had been hypothesized that to achieve maximum benefits from antithrombin in severe sepsis, a blood level of 200–250% of normal levels was necessary [12,13]. It was anticipated that the dose of antithrombin in the KyberSept trial would achieve target blood levels since a previous clinical trial using the same dosage in patients with severe sepsis achieved antithrombin plasma levels of about 200% over 4 days of treatment [61]. The possibility that the dose used in the KyberSept trial was not in the range of the weight-adjusted dose used in those animal studies that demonstrated improvement, might have played a role in the outcome difference. These differences in trial design, entry criteria, and baseline characteristics may have contributed to the differences in Phase III trial results, and led to the failure of the KyberSept trial.

Subgroup analysis
In the PROWESS trial, a consistent treatment effect was noted across all subgroups, following prospectively defined subgroup analyses for:
- average Acute Physiology and Chronic Health Evaluation II score
- number of dysfunctional organs or organ systems
- sex
- age
- site of infection
- type of infection
- pretreatment presence or absence of Activated Protein C deficiency.

In the KyberSept trial, the lack of effect was not consistent across all subgroups. The subgroup of antithrombin-treated patients without heparin has been previously discussed. Other patient groups fared better on subgroup analysis in regard to the change in relative risk; however, in all subgroups, confidence intervals did not reach statistical significance. In the antithrombin-treated patients, this nominal reduction in risk was associated with:
- lower antithrombin levels at baseline
- Gram-positive infection
- younger patients
- female patients
- high-risk classification by Simplified Acute Physiologic Scale II score
- absence of a surgical pathology.

The coordinating center concept
Another trial design difference that may have contributed to the failure of the KyberSept trial was the lack of an academic coordinating center. An integral part of the PROWESS trial was the necessity to obtain direct clearance for those patients who had malignancies or other potential exclusionary criteria. This coordinating center function was available 24 h a day, 7 days a week throughout the entire trial. Each patient with questions related to inclusion and exclusion criteria was specifically discussed with the coordinating center staff before the randomization process was initiated. This process was instituted to make certain that patients met entry criteria, and met the spirit, as well as the letter, of the trial protocol.

The KyberSept trial had a 24-h hot-line service to answer questions and discuss potential study patients. This was a voluntary service to KyberSept investigators and while this service was widely used, it was not mandatory for trial enrollment.

This coordinating center process may have significantly contributed to the favorable conclusion of the PROWESS trial. The need to discuss each patient before study entry is an excellent means to assure the predefined entry criteria of the trial are met, and to avoid randomization of patients that are unlikely to benefit from the intervention under trial. The degree to which this coordinating center contributed to the successful outcome of the PROWESS trial is difficult to quantify. It may have been of immeasurable benefit in the acquisition of a discriminatory study population for a pivotal Phase III trial in this complex patient population. It would seem reasonable to attempt to replicate this experience with clinical coordinating centers in future clinical trials of innovative agents in severe sepsis.
Summary and conclusion
Two contemporaneous Phase III sepsis trials with comparable endogenous human anticoagulants concluded with different results. The PROWESS trial of drotrecogin alfa (activated) delivered a long-awaited result of improved outcome in the saga of the development of new treatments for sepsis. The KyberSept trial with antithrombin did not demonstrate an improved outcome. The possible explanations for these disparate results are multiple. It seems, to begin with, that the patient populations in the two studies were different, as suggested by the mortality differences between placebo groups. It is likely that a variety of factors contributed to the Phase III results: mechanistic differences, trial design, entry criteria, and baseline characteristics, along with drug interactions with heparin.

There are mechanistic differences, despite similar physiological roles, for Activated Protein C and antithrombin. Activated Protein C has pro-fibrinolytic activity in vitro, which is not shared by antithrombin. Both compounds mediate anti-inflammatory effects, each by inhibition of thrombin (generation) and direct interaction with cells. Activated Protein C protects endothelial cells from apoptosis in experimental systems.

The entry criteria may have selected populations that were in different phases of sepsis; early (reversible) versus late (irreversible) sepsis. The continuous involvement of an academic coordinating center in the PROWESS trial may have aided in the selection of a more homogeneous study population and the adherence to protocol. Moreover, dosing strategies may have favored the PROWESS trial, as a weight-adjusted dose regimen was used in the PROWESS trial but not in the KyberSept trial. A weight-adjusted dosing plan may have increased levels of antithrombin activity to the expected, higher post-infusion levels, with corresponding therapeutic benefits.

Heparin interferes with the anti-inflammatory properties of antithrombin and promotes its systemic anticoagulant activities. The drug interaction between antithrombin and heparin was principally responsible for the increased bleeding events, reduced local anticoagulant activity, and the loss of antithrombin’s anti-inflammatory properties within the microcirculation.

The results of the PROWESS trial hold exciting promise for the treatment of severe sepsis. Additional agents would enrich the therapeutic armamentarium. Continued study in basic research should lead to a better understanding of the mechanistic differences between Activated Protein C and antithrombin. Future trials of antithrombin may show a more positive outcome if concomitant heparin therapy is avoided and a weight-adjusted dosing regimen is used.

References


