Protein C Replacement in Severe Meningococccemia: Rationale and Clinical Experience

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Severe meningococccemia, which is associated with hemodynamic instability, purpura fulminans and disseminated intravascular coagulation, still has a high mortality rate, and patients who survive are often left invalids because of amputations and organ failure. Clinical studies have shown that levels of protein C are markedly decreased in patients with severe meningococccemia and that the extent of the decrease correlates with a negative clinical outcome. There is a growing body of data demonstrating that activated protein C, in addition to being an anticoagulant, is also a physiologically relevant modulator of the inflammatory response. The dual function of protein C may be relevant to the treatment of individuals with severe meningococcal sepsis. In the present review we give a basic overview of the protein C pathway and its anticoagulant activity, and we summarize experimental data showing that activated protein C replacement therapy clearly reduces the mortality rate for fulminant meningococccemia.

The effect of invasion of the bloodstream by Neisseria meningitidis can vary from a transient, mild febrile illness to (in about 10% of cases) an acute fulminant disease that may be fatal within hours. Fulminant meningococccemia is characterized by profound endotoxinemia leading to vasomotor collapse, multiple organ failure, and disseminated intravascular coagulation. Clinical hallmarks are rapidly enlarging skin and mucosal hemorrhagic lesions (given the name “purpura fulminans”) and/or arterial thrombi leading to gangrene of digits and limbs. There is an increasing amount of experimental and clinical data indicating that infusion of protein C not only can reverse the procoagulant state but also can reduce the inflammatory reaction in fulminant meningococccemia. The present review describes the anticoagulant and anti-inflammatory action of activate protein C and summarizes the published clinical experience with protein C replacement in severe meningococccemia.

THE PROTEIN C PATHWAY

The 3 most important regulators of coagulation are (1) the tissue factor pathway inhibitor, which directly inhibits activated factor X (factor Xa) and, complexed to factor Xa, mediates a feedback inhibition on tissue factor and activated factor VII (factor VIIa); (2) antithrombin, which mainly inhibits thrombin and factor Xa; and (3) the protein C pathway (figure 1). Protein C becomes activated by thrombin bound to vascular
Protein C Replacement in Meningococcemia

Protein C (PC) activation and anticoagulant action of activated protein C. Upper panel: The tenase complex (formed by activated factor IX [IXa], activated factor VIII [VIIIa], calcium ions, and negatively charged membrane phospholipids) activates factor X. Activated factor X [Xa] then forms with activated factor V [Va], calcium ions, and a negatively charged phospholipid surface, the prothrombinase complex, which converts prothrombin (II) to thrombin (IIa). Thrombin can either promote clotting and activate cells or it can bind to thrombomodulin (TM), leading to PC conversion to the anticoagulant activated protein C (APC). PC activation by the thrombin-thrombomodulin complex is facilitated by the transmembrane protein endothelial protein C receptor (EPCR). Bottom panel: Protein S (PS) facilitates APC binding to cell surfaces and enhances APC-mediated cleavage of coagulant factors VIIIa and Va. The inactivated forms of these cofactors (VIIIi and Vi) are no longer capable of sustaining thrombin generation.

Human protein C is a vitamin K–dependent plasma glycoprotein, consisting of a light chain of 21 kd and a heavy chain of 41 kd, joined by a single disulfide bridge [1, 2]. The gene of protein C, spanning 12 kilobases and containing 9 exons, is located on chromosome 2. Protein C is synthesized by the liver as a single-chain glycoprotein, which is cleaved after secretion and circulates at a plasma concentration of ∼4 μg/mL. The light chain contains, in its N-terminal region, 9 posttranslationally γ-carboxylated glutamic-acid residues, which are necessary for further intracellular processing and for calcium-dependent binding to negatively charged membranes. Next to the vitamin K–dependent glutamic acid domain, there is a sequence rich in hydrophobic residues and 2 epidermal growth factor domains.

The serine protease domain is located in the heavy chain. Here, occupancy of a small calcium-binding site produces a conformational change that allows protein C to be readily activated by thrombin bound to vascular thrombomodulin but not by free thrombin. The major site of protein C activation is probably the microcirculation, where, because of a high ratio of endothelial cell surface to blood volume, the thrombomodulin concentration is ∼100 nM. The complex thrombin-thrombomodulin cleaves a single bond (Arg 12–Leu 13) at the N-terminal end of the heavy chain, thereby transforming the zymogen protein C to activated protein C, a serine protease with enhanced proteolytic activity.

Activated protein C rapidly dissociates from the thrombin-thrombomodulin complex and inactivates coagulant factor Va and factor VIIIa by cleaving specific Arg-containing peptide bonds. For instance, activated protein C cleaves factor Va first at Arg 506, which results in rapid but incomplete loss of activity, and subsequently at Arg 306, which leads to complete inactivation. A third cleavage site is at Arg 679. Simultaneously, activated protein C enhances the action of tissue plasminogen activator by inactivating its inhibitor plasminogen activator inhibitor 1, thereby stimulating the fibrinolytic system.

The action of activated protein C is potentiated by protein S. Human protein S is a single-chain vitamin K–dependent glycoprotein of 70 kd [1–3]. It is synthesized by hepatocytes, vascular endothelial cells, and megakaryocytes. In human plasma, protein S is present at a total concentration of 20–25 μg/mL and is found in at least 2 forms: ∼40% circulates as free protein and ∼60% as a noncovalent complex with a large (570-kd) multisubunit regulatory protein of the classic complement pathway, C4b-binding protein. Only free protein S is functionally active as an anticoagulant cofactor, although protein S complexed to C4b-binding protein retains its ability to interact with activated protein C and competitively inhibits the activity of the free form.
Protein S facilitates binding of activated protein C to platelet and endothelial cell surfaces. In addition, it enhances activated protein C–mediated inactivation of factor Va by promoting cleavage at Arg 306 and by abolishing the ability of factor Xa to protect factor Va. Similarly, protein S also enhances inactivation of factor VIIIa by blocking the ability of activated factor IX (factor IXa) to protect factor VIIIa from the proteolytic action of activated protein C.

Recently, an endothelial protein C receptor was identified [4, 5]. This is a transmembrane glycoprotein homologous to the major histocompatibility complex class I family of molecules [4, 6] and is mainly expressed on the surface of large vessels [7]. In vitro studies indicate that the major function of the cellular form of the endothelial protein C receptor is the facilitation of protein C activation by the thrombin-thrombomodulin complex [8], especially on large vessels where the concentration of thrombomodulin is low. It is intriguing that the soluble form of the endothelial protein C receptor inhibits the anticoagulant activity of activated protein C without altering its sensitivity to inhibition by protein C inhibitor or α1-antitrypsin [9]. This observation suggests that soluble endothelial protein C receptor may modulate the substrate specificity of activated protein C in a manner reminiscent of the influence of thrombomodulin on thrombin [9].

Three aspects of the protein C pathway deserve particular mention. (1) The pathway is activated by thrombin bound to vascular thrombomodulin. Such a mechanism is responsible for “on-demand” activation of protein C and therefore for an anticoagulant response whose magnitude is proportional to the level of thrombin generated [10]. (2) Thrombomodulin acts as a “molecular switch” for thrombin. Not only does thrombin that is bound to thrombomodulin efficiently activate an important anticoagulant pathway, but it also no longer functions as a procoagulant: it has a diminished ability to clot fibrinogen, to activate clotting factors such as factors V, VIII, and XIII, and to induce platelet activation [11]. Moreover, thrombin bound to thrombomodulin complexes more rapidly with antithrombin and protein C inhibitor than free thrombin does, and so is quickly inactivated. (3) Activated protein C has a half-life in circulation of ~15 min [12], demonstrating an unusual resistance to the action of serine protease inhibitors, such as protein C inhibitor and α1-antitrypsin (for comparison, thrombin has a half-life of 10–20 s). Its long half-life suggests that once activated protein C is generated, it can circulate throughout the vascular bed as a “sentry” and inactivate multiple Va and VIIIa molecules on membrane surfaces.

In summary, the protein C pathway is designed to block efficiently the procoagulant activity of thrombin, to inhibit the amplification of the coagulation response brought about by cofactors factor Va and factor VIIIa, and to stimulate endogenous fibrinolysis.

LINKS BETWEEN INFLAMMATION AND COAGULATION

The systemic inflammatory response that occurs in sepsis is generated by the interplay between several microbial and host-derived mediators. Bacterial endotoxin, which is composed of lipopolysaccharide, is a component of the outer membrane of gram-negative bacteria and is a powerful trigger of the host response. Bacterial membrane-bound and released lipopolysaccharide can interact with a variety of lipophilic proteins. The end results of lipopolysaccharide action are complement activation generating the membrane attack complex C5b9 [13, 14] and synthesis of inflammatory mediators, including platelet-activating factor and an array of proinflammatory cytokines [15]. In humans, the most avid lipopolysaccharide receptor is CD14 [15], which is found on cells such as monocytes, macrophages, and neutrophils.

Two endogenous monocyte/macrophage-derived cytokines, TNF-α and IL-1β, play a major role in the development of the inflammatory host response [16, 17]. The cytokine system functions as a network of communication signals between neutrophils, monocytes, macrophages, and endothelial cells to potentiate the inflammatory response once it is activated by a systemic microbial challenge (e.g., endotoxemia). If regulatory control is lost, the inflammatory response results in diffuse endothelial injury, septic shock, and multiple organ dysfunction.

A characteristic complication of sepsis is activation of coagulation, leading in the most severe cases to a consumptive coagulopathy and diffuse thrombi in the microcirculation [18] and resulting in purpura-like lesions similar to those in infants with homozygous protein C deficiency [19]. Challenge of healthy volunteers with lipopolysaccharide and TNF-α indicates that the extrinsic pathway is the predominant mechanism by which the coagulation system is activated in sepsis [20, 21]. Lipopolysaccharide and TNF-α can interact with monocytes, inducing synthesis and expression of tissue factor [22, 23], and both substances can promote endothelial expression of tissue factor in vitro [24, 25]. Exposure on the platelet surface of negatively charged aminophospholipids, which are critical for the assembly of tenase and prothrombinase complexes, can be brought about by the membrane attack complex C5b9 [26] and by the combined action of thrombin and exposed subendothelial collagen [27]. These mechanisms provide a trigger to initiate and amplify the coagulation response. In addition, recent publications indicate that circulating microparticles may have a critical role in the generation of a consumptive coagulopathy [28, 29].

In addition to the extrinsic coagulation pathway, the contact activation system, including factor XII, prekallikrein, and high–molecular-weight kininogen, is also activated. This initiates vasodilation by generating bradykinin from high–molecular-weight kininogen [20] and potentiates lipo-
polysaccharide-induced activation of the complement system [13] through activation of the complement component C1, mediated by activated factor XII (factor XIIa) [30].

At the same time, the inflammatory response inhibits the anticoagulant system. Antithrombin becomes complexed with thrombin and other proteases, and activated protein C becomes complexed with protein C inhibitor and α1-antitrypsin, and both are thereby consumed. Furthermore, antithrombin acts as a negative acute-phase protein [31], and its synthesis is diminished [18]. TNF-α [32–34], IL-1β [35, 36], and lipopolysaccharide [25] can interact with the endothelium to down-regulate thrombomodulin, although the extent of this downregulation appears to be less in vivo than in vitro. In addition, activated neutrophils can decrease the function of endothelial thrombomodulin by releasing reactive oxygen species, which can oxidize a specific methionine on thrombomodulin critical for protein C activation [37], and by releasing elastase, which can cleave thrombomodulin [38]. These mechanisms lead to decreased thrombin inactivation and decreased generation of activated protein C. In addition, as a consequence of complement activation and cytokine elaboration, the serum level of C4b-binding protein increases, thus diminishing the availability of free protein S for supporting activated protein C [3]. Moreover, since one of the major inhibitors of activated protein C, α1-antitrypsin, is an acute-phase reactant, the rate of inhibition of activated protein C is increased [39]. Finally, the acute inflammatory response also raises the concentration of plasminogen activator inhibitor 1, decreasing fibrinolytic activity [40].

In summary, systemic inflammation disrupts the balance between procoagulant, anticoagulant and fibrinolytic systems, leading to a massive activation of intravascular coagulation, which results in microthrombi and depletion of coagulation factors [18]. Severe diffuse intravascular coagulation, associated with endothelial cell dysfunction and diffuse microvascular thrombosis, heralds a poor prognosis.

Why is a prothrombotic state favorable for the inflammatory response? Thrombin not only plays a role in clot formation and in triggering an anticoagulant response but also mediates cellular proliferation and inflammation [41, 42]. For instance, thrombin appears to be directly chemotactic for neutrophils [43] and promotes synthesis by endothelial cells of platelet-activating factor, a potent neutrophil agonist [44], and of IL-8, the most potent chemotactic molecule for neutrophils in vivo [45]. Thrombin is also chemotactic for monocytes [46], where it induces an increase in intracellular calcium [41] and synthesis of IL-6 and IL-8 [47]. On endothelial surfaces, thrombin causes the expression of P-selectin and E-selectin, which are critical for neutrophil and monocyte tethering and activation [45, 48]. Thrombin has also been implicated in facilitating increased capillary permeability [49]. Activated platelets induce IL-8 production by endothelial cells [50] and increase IL-1 and TNF-α secretion by monocytes [51]. Finally, factor Xa also may function as a mediator of acute inflammation in vivo [52]. Thus, the propagation of a procoagulant state appears to represent an amplification loop of the inflammatory response.

**PROTEIN C AS AN ANTI-INFLAMMATORY AGENT**

Animal studies have provided evidence that the protein C pathway, in addition to its anticoagulant function, plays an important role in regulating the host response to inflammation, particularly sepsis (figure 2). Initially it was observed that thrombin infusion at a dose of 0.5 U/kg/min significantly increased survival rates among dogs that were subsequently challenged with a lethal dose of endotoxin [53]. At first sight this appears paradoxical, because thrombin generation leads to diffuse intravascular coagulation, which contributes to the mortality associated with septic shock. However, it had previously been shown that extracorporeal circulation without added heparin generated an endogenous anticoagulant [54] and protected dogs against endotoxin shock [55].

Second, it had also been shown that low-level thrombin infusion leads to a net anticoagulant response due to the formation of activated protein C through the thrombin-thrombomodulin complex [56, 57]. Therefore, it was hypothesized that generation of activated protein C might be responsible for some protective effect against endotoxin-induced septic shock.

Activated protein C that was infused into baboons before or 2 h after administration of lethal doses of *Escherichia coli* prevented the expected coagulopathic, hepatotoxic, and lethal responses [58]. These results have been reproduced in studies that have used other in vivo models: studies investigating endotoxin-induced pulmonary edema in rats [59, 60] and endotoxin shock in rabbits [61]. When endogenous protein C activation in baboons was blocked with a monoclonal antibody, *E. coli* doses that normally induce only an acute inflammatory reaction (10% of the lethal dose) caused a lethal septic shock response, which could be prevented by infusing activated protein C [58]. Similarly, blocking protein S function in baboons with an infusion of C4b-binding protein also exacerbated the response to sublethal concentrations of *E. coli*, and this could be prevented by infusing free protein S [62, 63].

It is noteworthy that the concentration of activated protein C that exhibited an anti-inflammatory effect was less than the concentration required for efficient anticoagulation [58, 59]. Moreover, the administration of other anticoagulants, such as heparin, alone or in combination with antithrombin, and active-site-blocked factor Xa (a powerful inhibitor of thrombin generation) inhibited endotoxin-induced coagulopathy but did not prevent shock and organ damage, nor did it improve the
Figure 2. Anti-inflammatory action of activated protein C. Activated protein C has the potential for regulating the inflammatory response by means of at least 3 mechanisms: (1) it prevents thrombus formation and stimulates fibrinolysis, thereby diminishing ischemic tissue damage; (2) it blocks thrombin generation, thereby preventing amplification of the inflammatory response induced by thrombin itself; and (3) it has a direct effect on monocytes that dampens elaboration of cytokines, such as TNF-α, IL-6, and IL-8. FVa, activated factor V; FVIIIa, activated factor VIII; PAI, plasminogen activator inhibitor.

The ability of activated protein C to regulate the inflammatory response seems to be related to a direct effect on monocytes. These cells have specific binding sites for activated protein C [68], which appear to be distinct from the endothelial protein C receptor [4]. In vitro studies have shown that activated protein C, in conjunction with protein S, reduces endotoxin-induced cytokine production by monocytes by >90% [69]. Pretreatment with activated protein C blocks the IFN-γ-induced increase in the amount of free intracellular calcium [68] and the activation of monocytes [69] and inhibits the monocyte-dependent proliferation of T cells [68]. In addition, activated protein C inhibits the CD14-dependent endotoxin-induced pathway of monocyte activation but does not prevent upregulation of the levels of major histocompatibility complex class II, intercellular adhesion molecule 1, or IL-2 receptor and does not prevent production of reactive oxygen intermediates [69]. These observations suggest that activated protein C has a differential anti-inflammatory action.

Although lipopolysaccharide and TNF-α downregulate thrombomodulin levels on endothelial cells, they induce increased cytosolic mRNA and surface thrombomodulin levels on monocytic cells [70, 71]. This provides the potential for localized monocyte-mediated production of activated protein C at sites of inflammation, even when thrombomodulin levels on endothelium have been downregulated. Another candidate receptor for preferential protein C activation in inflammation is the endothelial protein C receptor [72]. In a rodent model it has been demonstrated that lipopolysaccharide induces upregulation of levels of endothelial protein C receptor mRNA and that this is mediated by thrombin [73]. Moreover, the in vivo contribution of the endothelial protein C receptor to the negative regulation of coagulopathic and inflammatory responses to E. coli has recently been demonstrated [74].

The differential action of activated protein C on monocytes and macrophages—inhibiting the production of cytokines but maintaining the responses that are associated with adhesion, phagocytosis, and killing of gram-negative bacteria [69]—suggests that it could be used to treat inflammatory states that
involves activation of monocytes and/or macrophages and overproduction of cytokines, such as gram-negative sepsis.

**Protein C in Meningococcemia**

*Neisseria meningitidis* is an encapsulated aerobic gram-negative diplococcus. It colonizes the nasopharynx and causes infection by penetrating the mucosal barrier and entering the intravascular space. Meningococcemia varies from a transient, mild febrile illness to an acute fulminating disease that is fatal within hours [75]. It is not known which factors predispose to the development of the severe form, which is characterized by hemodynamic instability, disseminated intravascular coagulopathy, and diffuse microvascular thrombosis. However, clinical studies have found that increased levels of plasminogen activator inhibitor 1 [76] and decreased levels of protein C correlate with the development of purpura-like skin lesions and with a poor prognosis [77, 78]. It is particularly noteworthy that protein C activity was found to be decreased to a greater extent than was the activity of antithrombin or protein S, approaching levels similar to those observed in homozygous protein C deficiency [78].

Purified protein C concentrate is the first choice for therapy in cases of homozygous protein C deficiency with neonatal purpura [79–81] and has been successfully administered to patients with disseminated intravascular coagulopathy [82]. Gerson et al. [83] described the reversal of disseminated intravascular coagulopathy and purpura fulminans following administration of protein C concentrate in a child with septic shock who did not respond to aggressive conventional treatment.

Rivard et al. [84] described 2 girls and 2 boys (aged 3 months to 15 years) who were admitted to an intensive care unit with clinical findings of meningococcemia and purpura fulminans; results of laboratory studies revealed disseminated intravascular coagulopathy and protein C levels <0.5 IU/mL (normal level, 0.7–1.2 IU/mL). Aggressive conventional treatment was initiated with antibiotics, fluid resuscitation, vasoactive amines, and mechanical ventilation when required. Protein C was administered iv at a dose of 100 IU/kg for 15–20 min. Identical doses were given every 6 h during the acute phase. All 4 patients survived. However, 1 patient required bilateral mid-thigh and right mid-forearm amputations, as well as skin grafts on her left breast, and 1 patient required bilateral submalleolar amputation. It is noteworthy that both patients received protein C concentrate at a relatively late stage, 20 and 14 h, respectively, after the onset of skin lesions (vs. 7 and 8 h for the other 2 patients). Rintala et al. [85] described 3 more patients with meningococcemia, purpura fulminans, and multiple organ failure whose treatment included administration of protein C concentrate at a dosage of 100 IU/kg iv every 6–8 h. Laboratory and clinical parameters of coagulopathy and multiple organ failure improved. However, 1 patient died of cerebral edema.

Smith et al. [86] prospectively studied 12 patients (aged 3 months to 27 years) admitted to an intensive care unit with severe meningococcemia, septic shock, purpura fulminans, laboratory evidence of disseminated intravascular coagulopathy, and protein C levels <0.3 IU/mL. In addition to conventional treatment (with antibiotics, fluid resuscitation, inotropic drugs, and mechanical ventilation), all patients received continuous protein C concentrate infusion. After administration of a test dose (10 IU/kg), followed by a loading dose (100 IU/kg), protein C concentrate was continuously infused (10–15 IU/kg/h), with the aim of achieving a plasma concentration of 0.8–1.2 IU/mL. Additional treatment included unfractioned iv heparin (10–15 IU/kg/h) for 11 patients, hemodiafiltration for 9 patients, and peritoneal dialysis for 1 patient. All the patients survived. Two patients, who had received protein C concentrate later than the others (48 and 72 h after admission to the hospital, vs. ≤18 h for the other patients) needed lower-limb amputations; 1 of them also had a thrombotic cerebrovascular accident. This group of investigators has treated 30 patients thus far [87, 88]. Only the 2 above-mentioned patients who did not receive protein C replacement within 18 h after hospital admission required amputations. Three patients died (mortality rate, 10%), and the 25 who survived had minimal residual morbidity (2 required skin grafts and 1 has chronic renal failure that does not require dialysis) [87, 88].

Recently, Kreuz et al. described 8 children (aged 2 months to 18 years) with severe meningococcus-induced septic shock, purpura fulminans, disseminated intravascular coagulopathy, and acquired protein C deficiency [89, 90]. Six patients survived (1 required limb amputation), and 2 died. These results (6 deaths among 46 reported patients) compare favorably with an expected mortality rate of at least 30% to >50% for severe meningococcemia [77, 91–93]. In addition, administration of protein C halted the progression of skin lesions and disseminated intravascular coagulopathy and reduced the incidence of amputations, and in all these studies no adverse effects from protein C concentrate were noted.

**Conclusion**

Meningococcal sepsis is a fulminating disease requiring a high index of suspicion for diagnosis and immediate administration of antibiotics. Conventional therapy includes close observation, volume resuscitation, inotropic support, and early intubation [93, 94]. In addition, several experimental approaches have been proposed, such as plasmapheresis, antiendotoxin therapies, anticytokine therapies, use of heparin, and thrombolysis [93, 94]. There is an increasing amount of experimental and clinical data that strongly support the use of protein C replace-
ment in meningococcal purpura fulminans. The protein C pathway acts not only as an anticoagulant mechanism but also as an anti-inflammatory mechanism, and protein C replacement has been shown to improve the rate of survival and clinical outcome for patients with severe meningococcemia.

Of particular clinical interest is the fact that protein C replacement has been shown to be effective even when implemented several hours after hospital admission [86, 88] or after development of skin lesions [84]. Therefore, protein C replacement provides a valuable therapy for severe meningococcal disease. Protein C concentrate is not yet approved for clinical use, but it can be used in the context of clinical studies and may be available for compassionate use.

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Note Added in Proof While this manuscript was in press, 2 additional articles on protein C replacement therapy were published. White et al. (White B, Livingston W, Murphy C, Hodgson A, Rafferty M, Smith OP. An open-label study of the role of adjuvant hemostatic support with protein C replacement therapy in purpura fulminans-associated meningococcemia. Blood 2000; 96:3719–24) have updated their experience with protein C replacement in cases of severe meningococcemia [8688] to include 36 patients and report a mortality rate of 8%, which compares favorably with the predicted mortality rate of 50%. Bernard et al. (Bernard GR, Vincent JL, Laterre PF, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. N Engl J Med 2001; 344:699-709) have reported results from a randomized, double-blind, placebo-controlled, multicenter trial investigating whether iv administration of recombinant human activated protein C would reduce the death rate at 28 days among patients with severe sepsis of any cause. The data show that such treatment does significantly reduce mortality, but at the expense of an increased risk of bleeding.