Clinical and experimental studies on treatment of acute mesenteric ischemia
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Beyond sepsis: activated protein C and ischemia-reperfusion injury

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\textit{Future perspectives: anticoagulation benefits in ischemia and reperfusion syndromes}

Abstract

Objective: To review potential clinical situations beyond sepsis in which activated protein C might be effective.

Data Sources: Published articles on experimental and clinical studies of activation of both coagulation and inflammation in various disease states.

Data Synthesis/Conclusion: The efficacy of activated protein C in sepsis may rely on the fact that it can modulate both coagulation and inflammation. Therefore, a potential beneficial effect of activated protein C may be present in disease states that are also characterized by simultaneous activation of these systems. Ischemia-reperfusion injury of various organs may represent such a state. Indeed, in various experimental studies of ischemia-reperfusion, involvement of the protein C system was demonstrated. In some of these models a beneficial effect of administration of activated protein C, or other interventions in the protein C system, was shown.
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Introduction

Experimental studies indicate that an impaired function of the protein C pathway plays a major role in the pathogenesis of sepsis and associated organ dysfunction. Administration of activated protein C in a baboon model of intravenous E. coli resulted in survival of all animals whereas control animals in the same experiment all died. A similar beneficial effect was seen in rabbits with meningococcal endotoxin shock. Conversely, experiments in baboons in which the protein C pathway was blocked with monoclonal antibodies resulted in complete lethality of an otherwise sublethal bacteremia model. Also clinical trials in patients with sepsis have shown a beneficial effect of recombinant human activated protein C. Firstly, in a dose-ranging clinical trial, patients with sepsis received activated protein C at doses ranging from 12 μg/kg/hr to 30 μg/kg/hr, or placebo. Based on D-dimer plasma levels the optimal dose of recombinant human activated protein C was determined to be 24 μg/kg/hr. There was a clear trend towards a lower mortality (40% reduction) in patients receiving higher doses of activated protein C, although this was not statistically significant (due to the size of the trial). Subsequently, a large multicenter efficacy trial was prematurely stopped at the second interim analysis because of a significant reduction in mortality in the activated protein C-treated patients. Mortality was 24.7% in the activated protein C group as compared with 30.8% in the placebo group (relative risk reduction 19.4 percent, 95% confidence interval, 6.6 to 30.5). An extensive analysis of subgroups in this trial demonstrated that administration of recombinant human activated protein C proved to be of benefit in virtually all subgroups defined, including age, type and site of infection and disease severity. As expected, patients treated with activated protein C had less organ dysfunction. A recent analysis of long term survival of patients in this trial showed that the benefit of activated protein C was sustained over time.

Mechanisms of the beneficial effect of activated protein C in sepsis

Under physiologic conditions protein C is activated by thrombin bound to the endothelial cell membrane-associated thrombomodulin. Binding of protein C to thrombomodulin not only results in an about 100-fold increase in the activation of protein C, but also blocks the thrombin-mediated conversion of fibrinogen into fibrin and inhibits the binding of thrombin to other cellular receptors on platelets and inflammatory cells. Binding of protein C to the endothelial protein C receptor (EPCR) results in a further 5-fold augmentation of the activation of protein C by the thrombomodulin-thrombin complex. Dysfunction of the protein C system in sepsis is due to a combination of factors, including low levels of zymogen protein C (due to impaired synthesis and degradation by neutrophil elastase) and most notably by downregulation of thrombomodulin by pro-inflammatory cytokines. Observations in patients with severe Gram-negative septicemia indeed confirmed the downregulation of thrombomodulin and impaired activation of protein C in vivo.

Activated protein C is a physiological anticoagulant that modulates coagulation by proteolytically degrading the essential coagulation co-factors Va and VIIIa. Dysfunction of the protein C system may lead to inadequate balancing of tissue factor-mediated thrombin formation and may contribute to the formation of microvascular thrombosis, thereby deteriorating an adequate blood supply to various organs and be a factor in the...
occurrence of organ failure. Indeed, low levels of protein C in endotoxemic mice with a one allele targeted deletion of the protein C gene (resulting in a heterozygous protein C deficiency) resulted in more fibrin deposition. In addition, activated protein C may affect endogenous fibrinolysis by inhibiting the fibrinolytic inhibitor PAI-1. In a rat model of disseminated intravascular coagulation, activated protein C was shown to block PAI-1 activity and other experiments showed the ability of activated protein C to enhance clot lysis in vivo. The administration of activated protein C to patients with sepsis indeed results in a modulation of coagulation, as evidenced by a significant decrease in plasma D-dimer levels. Also, the presence of severe coagulation abnormalities in patients with sepsis (classified as disseminated intravascular coagulation) determines a subgroup of patients with the numerically largest benefit of activated protein C. Hence, there is ample evidence that activated protein C exerts its beneficial effects in sepsis through anticoagulant properties.

However, besides its anticoagulant effect activated protein C has important inflammation-modulating properties as well. Indeed, activated protein C has been found to inhibit endotoxin-induced production of TNF-α, IL-1β, IL-6 and IL-8 by cultured monocytes/macrophages. It is likely that the effects of activated protein C on inflammation are mediated by the endothelial protein C receptor (EPCR), that may mediate downstream inflammatory processes. Binding of activated protein C to the endothelial protein C receptor was shown to affect gene expression profiles of cells by inhibiting endotoxin-induced calcium fluxes in the cell and by blocking NFκB nuclear translocation, which is a prerequisite for increases in pro-inflammatory cytokines and adhesion molecules. Blocking the protein C pathway or the protein C receptor by a monoclonal antibody in septic baboons exacerbates the inflammatory response, as evidenced by increased levels of pro-inflammatory cytokines and more leukocyte infiltration and tissue destruction at histological analysis. Conversely, administration of activated protein C ameliorates the inflammatory activation in various models of severe systemic inflammation. Mice with a one-allele targeted disruption of the protein C gene have not only a more severe coagulation response to endotoxin but also demonstrate significant differences in inflammatory responses, as shown by higher levels of circulating pro-inflammatory cytokines. In the clinical trial in septic patients receiving activated protein C, interleukin-6 levels were significantly lower in the treatment group, although the result of other cytokine assays are less unequivocal. Nevertheless, inflammation-modulating properties of activated protein C may certainly be relevant for patients with sepsis.

It is not clear whether the beneficial effect of activated protein C in sepsis is either due to its anticoagulant or its anti-inflammatory effects. It should be remembered that the clinically effective dose of recombinant activated protein C was based on D-dimer levels in the phase II study, suggesting that the treatment is tailored to its anticoagulant effect. If, however, the anticoagulant effect would be most prominent, it is less clear why intervention in other physiological anticoagulant pathways in patients with sepsis was not effective. On the other hand, the significance of the inflammation-modulating properties of activated protein C in vivo are not completely clear and the anti-inflammatory effect of activated protein C in clinical trials is hard to assess. Of note, other potent anti-inflammatory agents were shown not to be effective in patients with sepsis in previous trials. Hence, the benefit of activated protein C is hard to explain on
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the basis of anticoagulant or anti-inflammatory effects exclusively. Instead, it is most likely that the beneficial effect is due to a combination of anticoagulant and anti-inflammatory properties. In view of the central role of endothelium in maintaining vascular patency and integrity, the strength of activated protein C may be its capacity to restore the deranged coagulant and inflammatory regulation at the endothelial site.

Potential benefit of activated protein C in other clinical settings

It is tempting to speculate that other clinical situations that are characterized by endothelial dysfunction and microvascular failure may benefit from the administration of recombinant activated protein C. Thereby, a prominent role of activated protein C may be envisaged in ischemia-reperfusion syndromes. Virtually all organs may suffer from ischemia-reperfusion injury, which can play an important role in major clinical entities, such as myocardial infarction, acute renal failure, stroke, acute lung injury and intestinal ischemia. Ischemia-reperfusion injury is characterized by a local inflammatory response and local activation of coagulation, reminiscent of the systemic situation in sepsis. Also, besides ischemia-induced cell necrosis, apoptosis may play a role as well. On all these mechanisms, activated protein C may have an effect. In the following we will briefly review the involvement of the protein C system in a selected number of models of ischemia-reperfusion injury. Other potential areas besides ischemia-reperfusion injury-related disease where activated protein C may be effective (such as stroke and other neurological disease, pancreatitis, arterial and venous thromboembolism) fall beyond the scope of this article and will not be discussed.

Role of the protein C system in ischemia-reperfusion injury

Renal ischemia-reperfusion syndromes are characterized by tubular necrosis and glomerular thrombosis. An important role of the protein C system in preventing glomerular thrombosis may be inferred from the abundant presence of thrombomodulin expression on endothelial cells in the glomerulus. In inflammatory glomerular disease, such as acute membranoproliferative or lupus glomerulonephritis, an increase in thrombomodulin expression has been implicated. In contrast, in ischemia-reperfusion injury in kidneys, thrombomodulin has been markedly downregulated. Administration of soluble thrombomodulin to rats with renal ischemia-reperfusion injury prevented massive glomerular thrombosis and kidney dysfunction. In another experimental study of renal ischemia and reperfusion administration of activated protein C prevented histological changes and the decrease in renal blood flow, and preserved kidney function, whereas treatment with active site-blocked factor Xa, heparin and inactivated protein C were less effective. Interestingly, a significant effect of ischemia-reperfusion-associated inflammation was observed upon the administration of activated protein C. The increase in renal levels of inflammatory cytokines TNF-α and IL-8 and renal myeloperoxidase activity was significantly reduced in activated protein C-treated animals whereas other interventions had no such effect. Furthermore, in an experimental model of liver ischemia and reperfusion, a similar anti-inflammatory responsiveness was demonstrated. Activated protein C, but also active human urinary thrombomodulin significantly reduced the production of cytokine-induced neutrophil chemoattractant and decreased myeloperoxidase activity and leukocyte accumulation, resulting in attenuation of liver...
injury following ischemia and reperfusion. The notion that activated protein C had anti-inflammatory effects in these models of ischemia-reperfusion was supported by the observation that renal injury could also be prevented in rats that were severely leukocytopenic during the experiment. It is interesting to note that in sepsis-induced renal changes, no effect on thrombomodulin expression and protein C activation was found.

In coronary arteries of patients with severe atherosclerosis a marked downregulation of thrombomodulin and the protein C receptor was demonstrated on endothelial cells overlying the atherosclerotic plaque, suggesting a role of the protein C system in acute coronary syndromes. Interestingly, administration of activated protein C prolonged the time to occlusion and improved vessel patency and myocardial blood flow in a canine model of coronary artery thrombosis and reperfusion. In coronary artery reperfusion during heart surgery, activation of protein C was shown to be related to regulation of inflammatory activity. Together, it is likely that the protein C system may play a role in coronary artery syndromes and myocardial reperfusion injury.

In acute lung injury the abundant presence of intra- and extravascular fibrin is a histological hallmark. Experimental and clinical studies have shown that fibrin deposition is due to tissue factor-mediated thrombin generation and suppressed fibrinolysis. In recent experiments, activation of bronchoalveolar coagulation in severe pneumonia was shown to be restricted to the site of acute lung injury. In these same series of experiments, a significant reduction in bronchoalveolar protein C activation was observed, as evidenced by low levels of protein C and activated protein C in lavage fluids from patients with community acquired and ventilator-associated pneumonia. Similar findings were reported in a study in patients with acute lung injury and adult respiratory distress syndrome (ARDS). The decrease in protein C activation in both studies was strongly correlated with high levels of soluble thrombomodulin in the bronchoalveolar fluid, suggesting that shedding of thrombomodulin caused the inability to activate protein C and implicating that the protein C system may be involved in acute lung injury as well.

Acute intestinal ischemia and reperfusion may result in impaired intestinal structure and function, in experimental models characterized by intestinal cell swelling and protein leakage and impaired intestinal absorptive capacity. In addition, intra- and extravascular fibrin deposits may be present, due to activation of mesenteric coagulation and inhibition of fibrinolysis. Upon 20 to 40 minutes occlusion of the superior mesenteric artery and subsequent reperfusion, portal vein plasma levels of thrombin-antithrombin levels increased, indicating local thrombin generation. This increase in portal coagulation activity is associated with a marked fall in protein C activity levels. Simultaneously, markers for fibrinolysis in portal plasma showed a complete inhibition, due to an increase in levels of plasminogen activator inhibitor, type 1 (PAI-1). This activation of coagulation upon ischemia-reperfusion could be almost completely blocked by systemic administration of activated protein C, whereas heparin and antithrombin were less effective. Interestingly, amelioration of ischemia-reperfusion-induced intestinal intra- and extravascular fibrin deposition by administration of activated protein C caused a significant improvement in intestinal function and structure.

Taken together, there is interesting evidence to support a role of the protein C system in ischemia-reperfusion injury. Consequently, administration of activated protein C may be a
promising therapeutic option in these situations. The efficacy of the approach deserves further study in experimental and clinical studies.
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