Patients with severe sepsis vary markedly in their ability to generate activated protein C

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Activated protein C (APC) supplementation significantly reduces mortality in patients with severe sepsis, presumably by down-regulating coagulation, inflammation, and apoptosis. In vivo, endogenous APC is generated from protein C (PC) “on demand” in response to elevated thrombin levels. Thrombomodulin and endothelial cell protein C receptor are endothelial receptors required to generate APC endogenously. Since these receptors may be down-regulated in sepsis, we measured plasma markers of APC generation in 32 patients with severe sepsis to determine whether APC generation is impaired and whether markers of APC generation correlate with 28-day mortality. Relative to normals, all patients had elevated F1 + 2 and thrombin-antithrombin complex (TAT) levels (markers of thrombin generation and inhibition, respectively), and 28 of 32 patients had reduced PC levels. In 20 patients, APC levels paralleled elevated F1 + 2 levels, whereas 12 patients had low APC levels despite elevated F1 + 2 levels, suggesting that APC generation is impaired in the latter. No significant differences exist between survivors and non-survivors with respect to baseline PC levels, F1 + 2 levels, and APACHE II (acute physiology and chronic health evaluation) scores. Baseline APC levels were higher in survivors (P = .024), and baseline F1 + 2/PC ratios were lower in survivors (P = .047). Larger studies are warranted to establish whether APC generation profiles aid in managing sepsis.

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Introduction

Sepsis is a devastating disorder characterized by systemic activation of the inflammatory and coagulation cascades in response to microbial infection.1 Sepsis is defined as systemic inflammatory response syndrome (SIRS) in the presence of documented or suspected infection.2 When sepsis is associated with acute organ dysfunction, the sepsis is considered severe.2 In the United States, approximately 750 000 episodes of severe sepsis occur each year, of which 215 000 result in death,3 a value similar to the number of deaths due to acute myocardial infarction.4 The mortality rate is approximately 30% and increases to 40% in the elderly,3 and the incidence of severe sepsis is projected to increase by 1.5% per annum due to the growing proportion of elderly persons in society.3

Over the past 15 years, many treatments for sepsis have shown early promise yet failed to improve survival in septic patients. These therapies were directed at treating sepsis largely through attenuation of inflammatory mediators or by neutralization of endotoxin.5 It is now well accepted that inflammation, coagulation, and apoptosis occur concomitantly in sepsis and are intimately linked. Recently, a large phase 3 multinational placebo-controlled randomized clinical trial demonstrated the efficacy and safety of recombinant activated protein C (APC) for severe sepsis.6 Compared with placebo, a 4-day infusion of recombinant APC resulted in a reduction in the relative risk of death of 19.4% and an absolute reduction in the risk of death of 6.1% (P = .005). This was the first randomized trial to demonstrate a survival benefit in the treatment of severe sepsis.

The protective effect of APC supplementation in patients with severe sepsis likely reflects the ability of APC to modulate multiple pathways. APC, an endogenous plasma enzyme, is well established as a physiologically important anticoagulant.7,8 In addition to its anticoagulant properties, APC is profibrinolytic by inactivation of plasminogen activator inhibitor (PAI-1).9-11 APC also possesses anti-inflammatory properties. In vitro, APC down-regulates production of proinflammatory cytokines from monocytes12-14 and suppresses expression of leukocyte adhesion molecules in endothelial cells.15 In animals challenged with endotoxin, APC inhibits the production of inflammatory cytokines16,17 and inhibits leukocyte adhesion.18 Finally, APC has been shown to inhibit apoptosis of endothelial cells15,19 and to exert a neuroprotective effect in a murine model of focal ischemic stroke.20

In vivo, endogenous APC is generated in the circulation “on demand” from its inactive precursor protein C.7 The signal that triggers the conversion of protein C to APC is thrombin. Briefly, vascular injury or endotoxin/inflammatory cytokines initiates the coagulation cascade, ultimately resulting in thrombin generation and blood clot formation. Excess thrombin then complexes with...
thrombomodulin (TM), a receptor on the surface of vascular endothelial cells. The thrombin-TM complex rapidly converts protein C to its active form APC. APC generation is augmented by the endothelial cell protein C receptor (EPCR), which binds circulating protein C and presents it to the thrombin-thrombomodulin complex.

In healthy, nonhuman primates, the amount of APC generated in vivo is proportional to thrombin levels, based on measurements of endogenous APC levels following a low-dose infusion of thrombin. Since TM and EPCR are endothelial receptors that are part of the “machinery” required to generate APC in response to elevations in thrombin levels, it has been hypothesized that down-regulation of EPCR and TM in pathologic conditions where there is systemic endothelial dysfunction may potentially impair the ability to generate APC despite high thrombin levels. In vitro studies of endothelial cells have shown that both TM and EPCR are down-regulated by inflammatory cytokines, including tumor necrosis factor-α and interleukin-1β. In children with meningococcal sepsis, levels of TM and EPCR in the endothelium of the dermal vasculature are lower than those of controls.

Although studies of endothelial cells are important in quantifying the expression of TM and EPCR under normal and pathologic conditions, these studies provide only a partial picture of the functional status of the protein C pathway in the systemic circulation. Studies of circulating plasma markers—particularly the levels of protein C, thrombin, and APC—could be helpful in determining whether the ability to generate APC in response to elevated thrombin levels in vivo is actually impaired. In this study, we examined plasma markers of endogenous APC generation in 32 adult patients with severe sepsis. The specific objectives of our study were to determine whether the ability to generate APC in response to elevated thrombin levels is compromised in patients with severe sepsis and to explore the hypothesis that markers of endogenous APC generation correlate with 28-day all-cause mortality in patients with severe sepsis. A unique feature of our study is that we have a newly developed APC assay that, for the first time, permits accurate and rapid measurements of plasma APC levels.

Patients and methods

Study population

We identified 32 patients with severe sepsis in the intensive care units (ICUs) of the Henderson General Hospital and the Hamilton General Hospital (Hamilton, ON, Canada) between March 2001 and May 2002. These are academic, tertiary care, university-affiliated, closed-unit ICUs. We collected baseline characteristics of the patients, including demographic information, acute physiologic, and chronic health evaluation (APACHE II) admission scores, multiple organ dysfunction (MOD) admission scores, comorbidities, organ function, site and type of infection, and hematologic tests.

Patients with severe sepsis were identified using the inclusion criteria described in Bernard et al. Infection criteria. Patients had to have a known infection or a suspected infection, as evidenced by one or more of the following: white blood cells in a normally sterile body fluid; perforated viscus, radiographic evidence of pneumonia in association with the production of purulent sputum; and a syndrome associated with a high risk of infection (eg, ascending cholangitis).

Modified SIRS criteria. Patients had to meet at least 3 of the following 4 criteria: a core temperature of at least 38°C (100.4°F) or at or below 36°C (96.8°F); a heart rate of at least 90 beats per minute, except in patients with a medical condition known to increase the heart rate or those receiving treatment that would prevent tachycardia; a respiratory rate of at least 20 breaths per minute or a PaCO₂ of no more than 32 mmHg or the use of mechanical ventilation for an acute respiratory process; a white blood cell count of at least 12 000/mm³ or no more than 4000/mm³ or a differential count showing more than 10% immature neutrophils.

Criteria for dysfunctional organs or systems. Patients had to meet at least 1 of the following 5 criteria. For cardiovascular system dysfunction, the arterial systolic blood pressure had to be no more than 90 mmHg or the mean arterial pressure of no more than 70 mmHg for at least 1 hour or despite adequate fluid resuscitation, adequate intravascular volume status, or the use of vasopressors in an attempt to maintain a systolic blood pressure of at least 90 mmHg or a mean arterial pressure of at least 70 mmHg; for kidney dysfunction, urine output had to be less than 0.5 mL per kilogram of body weight per hour for 1 hour, despite adequate fluid resuscitation; for respiratory-system dysfunction, the ratio of PaO₂ to FiO₂ had to be no more than 250 in the presence of other dysfunctional organs or systems or no more than 200 if the lung was the only dysfunctional organ; for hematologic dysfunction, the platelet count had to be less than 80 000/mm³ or to have decreased by 50% in the 3 days preceding enrollment; in the case of unexplained metabolic acidosis, the pH had to be no more than 7.30 or the base deficit had to be at least 5.0 mL in association with a plasma lactate level that was more than 1.5 times the upper limit of the normal value for the reporting laboratory. The exclusion criteria used in this study were pregnancy or breast-feeding, age younger than 18 years, use of unfractionated heparin to treat an active thrombotic event within 8 hours of blood sampling, and use of low-molecular-weight heparin at a dose higher than recommended for prophylactic use within 12 hours of blood sampling.

We also recruited 11 healthy adult volunteers from the Henderson Research Centre in September 2001. The volunteers were not receiving any medication at the time of blood sampling. No attempt to match cases and controls was made.

Blood collection

Blood was collected from patients with severe sepsis within 24 hours of meeting the definition of severe sepsis. Venous blood (9 mL) was drawn via indwelling catheters from the patients. As controls, venous blood (9 mL) was drawn via venipuncture from 11 healthy adult volunteers. For each patient or volunteer, 4.5 mL of the collected blood was immediately transferred into 15-mL polystyrene tubes containing 0.5 mL 0.105 M buffered trisodium citrate (pH 5.4), and the remaining 4.5 mL was transferred into 15-mL polystyrene tubes containing 0.5 mL 0.105 M buffered trisodium citrate (pH 5.4) and 100 μL of 1 M benzamidine HCl (ie, 20 mM benzamidine final). The blood was spun at 1500g for 10 minutes at 20°C, and the plasma was stored as aliquots at −80°C. The citrated plasma samples are used for protein C antigen assays, F1 + 2 assays, TAT (thrombin-antithrombin) complex assays, o1-antitrypsin assays, soluble EPCR assays, and soluble TM assays, whereas the citrate/benzamidine plasma samples are used for the APC assays. Benzamidine HCl, a reversible inhibitor of trypsinlike proteases including APC, is necessary at the time of blood collection to block irreversible inhibition of APC by plasma protease inhibitors. The benzamidine is removed during the APC assay wash steps, thus restoring the enzymatic activity of APC toward chromogenic substrates.

APC assay

The APC assay is performed as described in Liaw et al. Briefly, 96-well flat-bottom polystyrene microtiter plates (Dynex Technologies, Chantilly, VA) were coated with 100 μL of 5 μg/mL HAPC 1555 (a Ca²⁺-dependent, APC-specific monoclonal antibody) in coating buffer (20 mM HEPES [N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid], pH 7.5, 150 mM NaCl, 5 mM CaCl₂) for 2 hours at 37°C. The plates were then blocked for 1 hour at 37°C with 200 μL of blocking buffer (coating buffer containing 1% bovine serum albumin [BSA]). Since HAPC 1555 is a Ca²⁺-dependent antibody, prior to the APC assays the buffered trisodium citrate/benzamidine-containing plasma samples were anticoagulated and recalcified by supplementing with heparin, HEPES pH 7.5, and CaCl₂ to final concentrations of 2 U/mL, 20 mM, and 10 mM, respectively. Plasma samples (100 μL) were then transferred to HAPC 1555-coated microtiter plates and incubated at room temperature for 30 minutes. The wells were washed (2 × 10 minutes).
**Table 1. Baseline characteristics of 32 patients with severe sepsis**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>67 ± 13 (40, 87)</td>
</tr>
<tr>
<td>Gender, % female (no./total)</td>
<td>25.0 (8/32)</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>20.9 ± 6.8 (8, 37)</td>
</tr>
<tr>
<td>MOPS score</td>
<td>8.4 ± 4.0 (2, 16)</td>
</tr>
<tr>
<td>Primary site of infection, no.</td>
<td>19 Abdomen, 4 Urinary tract, 4 Other, 1 Unknown</td>
</tr>
<tr>
<td>Positive blood cultures, no.</td>
<td>9 Gram-negative bacteria, 9 Gram-positive bacteria, 4 Fungus, 10 Mixed</td>
</tr>
</tbody>
</table>

SD indicates approximate standard deviation; min, minimum; max, maximum; APACHE II, acute physiology and chronic health evaluation II score; and MOPS, multiple organ dysfunction score.

with wash buffer (coating buffer containing 0.05% Tween-20), and the chromogenic activity of bound APC was determined by the addition of 100 µL Spectrozyme PCs (0.5 mM) (Chromogenix, Milan, Italy) in coating buffer. The plates were placed at 37°C, and substrate hydrolysis was monitored at 405 nm in end-point mode over time using a Spectromax plate reader (Molecular Devices, Ramsey, MN).

A standard curve for the quantitative measurement of APC in plasma was generated as follows. Increasing amounts of APC (from 0 to 100 ng/mL) were spiked into 20 mM HEPES, pH 7.5, 2 U/mL heparin, 20 mM benzamidine (Sigma Aldrich, St Louis, MO), 5 mM CaCl2, and 1% BSA. Heparin and benzamidine are included in the APC samples to mimic the conditions present in the plasma samples. 100-µL samples were transferred to microtiter wells containing immobilized HAPC 1555 and incubated at room temperature for 30 minutes. The wells were washed (2 × 10 minutes) with wash buffer (coating buffer containing 0.05% Tween-20), and the chromogenic activity of bound APC was determined by the addition of 100 µL Spectrozyme PCs (0.5 mM) in coating buffer. The plates were placed at 37°C, and substrate hydrolysis was monitored at 405 nm in end-point mode over time.

**Assays for protein C, α1-antitrypsin, F1 + 2, and TAT**

Total protein C antigen and α1-antitrypsin antigen in citrated plasma samples was quantified by a sandwich-type enzyme immunoassay (Affinity Biological, Ancaster, ON, Canada). Levels of F1 + 2 and TAT in citrated plasma samples were quantified by the Enzygnost F1 + 2 micro kit and the Enzygnost TAT micro kit, respectively (Dade Behring, Germany).

**Assays for soluble TM and soluble EPCR**

Levels of soluble TM (sTM) in citrated plasma samples were quantified by sandwich-type enzyme immunoassays as previously described, except that the coating antibody was CTM 1009, and the detecting antibody was goat anti–mouse no. 261 polyclonal antibody. Levels of soluble EPCR (sEPCR) in citrated plasma samples were quantified by sandwich-type enzyme immunoassays as previously described, except that the coating and detecting antibodies were JRK 1535 and JRK 1495, respectively.

**Results**

**Study population**

We studied 32 adult patients with severe sepsis between March 2001 and May 2002. None of these patients received recombinant APC therapy since the drug was not approved for use in Canada until 2003. The patients ranged in age from 40 to 87 years (mean, 67.1; SD, 13.0), and 8 (25%) were female. The mean admission APACHE II score was 20.9 (SD, 6.8), and the mean MOD score was 8.4 (SD, 4.0). The overall 28-day mortality rate was 56.3%. Baseline characteristics of these patients are summarized in Table 1. The mean age of the 11 healthy volunteers was 33.5 years (SD, 8.5), and 4 (36.4%) were female. The identification of nonseptic, age- and sex-matched controls was beyond the scope of this study, since our primary goal was to compare surviving and nonsurviving septic patients, rather than to compare such patients to a normal range in healthy, matched control patients.

**Plasma levels of protein C, F1 + 2, TAT, APC, and α1-antitrypsin**

Plasma markers of protein C activation (ie, protein C, F1 + 2, TAT, and APC levels) and APC inhibition (α1-antitrypsin levels) were measured in both septic patients and in healthy volunteers. The mean levels of these markers in the septic patients and in the healthy volunteers are summarized in Table 2. As shown in Figure 1, relative to normal pooled plasma, the plasma from all 32 septic patients had elevated F1 + 2 levels (indicative of thrombin generation), and 28 of the 32 patients had reduced protein C levels. Relative to normal pooled plasma, all septic patients also had elevated levels of TAT, a marker of thrombin inhibition (Table 2).

The patients appear to fall into 2 categories in terms of their ability to generate APC in response to elevated thrombin levels. In Canada. Informed consent was obtained from the patient/subject decision-maker prior to blood collection. All data were confidential.

**Statistical methods**

Baseline age, APACHE II and MOD scores, and protein C, F1 + 2, TAT, APC, α1-AT, sEPCR, and sTM levels for sepsis patients were summarized using means, standard deviations, minimums, and maximums. In addition, the latter 7 markers also were summarized for the healthy volunteers. Baseline APACHE II scores, protein C, F1 + 2, APC, and ratios of F1 + 2 to APC for survivor and nonsurvivor patients were compared using the Wilcoxon rank sum test. All tests were 2-sided, and statistical significance was defined as P values less than .05. Since this investigation was exploratory, no adjustments were made for multiple testing. All group comparisons were presented graphically using dot plots. Pearson correlation coefficients were used to assess the degree of the linear relationship between the continuous variables.

**Table 2. Base-line markers of protein C activation and inhibition**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Severe sepsis (n = 32)</th>
<th>Healthy volunteers (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Min, max</td>
</tr>
<tr>
<td>Protein C (U/mL)</td>
<td>0.48 (0.23)</td>
<td>0.15, 0.97</td>
</tr>
<tr>
<td>F1 + 2 (nm)</td>
<td>1.99 (1.00)</td>
<td>0.77, 5.81</td>
</tr>
<tr>
<td>TAT (µg/L)</td>
<td>12.6 (11.1)</td>
<td>3.9, 42.6</td>
</tr>
<tr>
<td>APC (ng/mL)</td>
<td>4.36 (2.06)</td>
<td>0.71, 9.21</td>
</tr>
<tr>
<td>α1-AT (U/mL)</td>
<td>1.10 (0.21)</td>
<td>0.76, 1.30</td>
</tr>
</tbody>
</table>

Data are summarized using means, standard deviations (SD), minimums (min), and maximums (max).
Correlation of markers of APC generation with clinical outcome

Since excessive and sustained systemic injury of vascular endothelial cells in severe sepsis may ultimately lead to organ failure, we explored the hypothesis that the capacity to convert endogenous protein C to APC correlates with 28-day mortality. As shown in Figure 2, there were no statistically significant differences between survivors and nonsurvivors with respect to baseline APACHE II scores, protein C levels, and F1 + 2 levels. In contrast, baseline APC levels in survivors (mean SD = 5.3 ± 2.2 ng/mL) were statistically significantly higher than in nonsurvivors (3.7 ± 1.6 ng/mL) (P = .024) (Table 3). In addition, baseline F1 + 2/APC ratios in survivors (0.57 ± 0.31) were significantly lower than in nonsurvivors (1.11 ± 0.80) (P = .047) (Table 3).

Plasma levels of soluble EPCR and soluble TM

In vitro studies of endothelial cells have shown that cell surface levels of EPCR and TM are down-regulated by inflammatory cytokines including tumor necrosis factor-α, β, IL-1β, and IL-10. The mechanisms by which these receptors are downregulated include inhibition of gene transcription and protease-mediated removal from the endothelium. The latter mechanism generates soluble forms of EPCR and TM, a process called "shedding." In this study, we measured plasma levels of soluble EPCR (sEPCR) and soluble TM (sTM) in septic patients to determine if increased circulating levels of these markers correlate with impaired protein C activation (measured by high F1 + 2-to-APC ratios) and/or disease severity (assessed by survival status at day 28). The mean levels of these markers in the septic patients and in the healthy volunteers are summarized in Table 4. Consistent with previous studies, sEPCR level in the septic patients (304.6 ± 98.2 ng/mL) were statistically significantly higher than in the healthy volunteers (mean, 212.4 ± 100.3 ng/mL) (P = .011), and the sTM levels in the septic patients (68.0 ± 258.2 ng/mL) and in the healthy volunteers (10.3 ± 21.0 ng/mL) were not statistically different (P = .494), although the variation in the septic patients was quite extreme. We observed no significant correlation between F1 + 2-to-APC ratios and sEPCR levels (r = 0.19, P = .29) and sTM levels (r = 0.10, P = .60). In addition, we observed a statistically significant difference between septic patients who survived and those who died with respect to sEPCR and sTM levels (P = .28 and P = .35, respectively).

Figure 1. Protein C, F1 + 2, APC, and α1-AT levels in 32 patients with severe sepsis. Plasma levels of protein C (PC), F1 + 2; APC, and α1-AT were measured in plasma as described in Patients and methods. The levels of these markers in the patients are expressed relative to normal pooled plasma (the green line indicates 100% of normal pooled plasma). The normal pooled plasma contains plasma pooled from 11 healthy volunteers. Nonsurviving patients are marked with an asterisk (*).

20 of the 32 patients, the plasma APC levels parallel the elevated F1 + 2 levels (Figure 1, top panel), whereas the remaining 12 patients have low APC levels despite elevated F1 + 2 levels (Figure 1, bottom panel). The identification numbers of the patients shown in Figure 1 do not reflect the sequential order in which the patients were recruited. Instead, the numbers are arbitrarily assigned to emphasize the observation that the patients vary markedly in their ability to generate APC endogenously. Since the protein C levels do not significantly correlate with the APC levels (r = 0.313, P = .081), the variations in APC levels are due not only to variations in protein C levels (Figure 1), but also likely reflect changes in the function of the protein C activation complex. The levels of α1-antitrypsin also do not significantly correlate with the APC levels (r = 0.001, P = .994), suggesting that variations in APC levels are not due to formation of α1-antitrypsin/APC inhibitory complexes (Figure 1).

Figure 2. Baseline APACHE II scores and plasma levels of protein C, F1 + 1, APC, and F1 + 2-to-APC ratios in survivors versus nonsurvivors. Baseline APACHE II scores (A), protein C levels (B), F1 + 2 levels (C), APC levels (D), and ratios of F1 + 2 to APC (E) for survivors and nonsurviving patients were compared using the Wilcoxon rank sum test. The means, standard deviations, minimums, and maximums of these parameters are summarized in Table 3.
Table 3. Base-line APACHE II scores and baseline markers of protein C activation and inhibition in survivors and in nonsurvivors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Survivors (n = 14)</th>
<th>Nonsurvivors (n = 18)</th>
<th>2-sided P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Min, max</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>APACHE II</td>
<td>19.8 (6.8)</td>
<td>8, 37</td>
<td>21.7 (6.9)</td>
</tr>
<tr>
<td>PC (U/mL)</td>
<td>0.52 (0.23)</td>
<td>0.15, 0.97</td>
<td>0.44 (0.23)</td>
</tr>
<tr>
<td>F1 + 2 (nM)</td>
<td>1.6 (0.6)</td>
<td>0.9, 2.6</td>
<td>2.2 (1.2)</td>
</tr>
<tr>
<td>APC (ng/mL)</td>
<td>5.27 (2.24)</td>
<td>1.54, 9.21</td>
<td>3.66 (1.64)</td>
</tr>
<tr>
<td>F1 + 2-to-APC ratio</td>
<td>0.57 (0.31)</td>
<td>0.20, 1.20</td>
<td>1.11 (0.80)</td>
</tr>
</tbody>
</table>

Baseline APACHE II scores and baseline markers of endogenous protein C activation for survivors and nonsurvivors were compared using the Wilcoxon rank sum test. Data are summarized using means, standard deviations (SD), minimums (min), and maximums (max).

Discussion

Recombinant APC is the first biologic agent shown to significantly reduce mortality in patients with severe sepsis. Part of the rationale for using APC to treat sepsis was that endogenous protein C levels drop rapidly in severe sepsis, attributed in part to increased conversion of protein C to its active form APC, and subsequent inactivation of APC by serine protease inhibitors such as α1-antitrypsin. The decrease in protein C levels can be rapid and often occurs hours before the onset of symptoms. In this observational study of 32 patients with severe sepsis, we measured circulating markers of endogenous protein C activation to address 2 important questions. First, is the ability to convert endogenous protein C to APC in response to elevated thrombin levels compromised in patients with severe sepsis? Second, do markers of endogenous APC generation correlate with 28-day mortality?

Although it is now well established that endogenous protein C levels are depressed in patients with severe sepsis, there are few available reports on endogenous APC levels in such patients, perhaps due to the lack of available assays that permit both rapid and accurate measurements. One study examined levels of APC in patients with severe sepsis treated either with recombinant APC or with placebo. The APC levels in the recombinant APC-treated group was approximately 45 ng/mL, whereas the APC levels in the placebo-treated group, which represents endogenous APC levels, were almost all (98.8%) nonquantifiable, since they were below the lower limit of detection of the assay (10 ng/mL). In another study, endogenous APC levels were monitored in patients with severe sepsis treated with placebo, and most (80%) had nonquantifiable APC levels below the assay limit of 5 ng/mL. Using our newly developed APC enzyme capture assay, which has a lower detection limit of 0.25 ng/mL and permits rapid detection of APC, we are the first to demonstrate that adult patients with severe sepsis vary markedly in their ability to convert endogenous protein C to APC in response to elevated thrombin levels. Based on the APC levels, the patients appear to fall into 2 categories in terms of their ability to generate APC: the first group has elevated APC levels that parallel the elevated F1 + 2 levels, whereas the second group has low APC levels despite elevated F1 + 2 levels (Figure 1). Since the APC levels do correlate with levels of protein C or α1-antitrypsin (Figure 1), impaired ability to generate APC in a subpopulation of adult septic patients may reflect systemic endothelial dysfunction, resulting in down-regulation of TM and EPCR levels on injured endothelium.

Down-regulation of cell surface TM and EPCR levels on injured endothelium is presumed to reflect endothelial cell damage and may occur by 2 distinct mechanisms: (1) via inhibition of TM and EPCR gene transcription, and (2) via protease-mediated shedding from the endothelial cell surface. The latter mechanism releases soluble forms of TM and EPCR into the circulation, whereas the former mechanism does not. In this study, we observed no correlation between high F1 + 2-to-APC ratios and elevated sEPCR levels nor between high F1 + 2-to-APC ratios and elevated sTM levels. In addition, we observed no statistically significant difference between septic patients who survived and those who expired with respect to sEPCR and sTM levels. These results suggest that a decrease in cell surface levels of TM and EPCR is a complex process and not only reflects shedding of the receptors but also decreases in TM and EPCR gene transcription.

Our observation that adult patients with severe sepsis vary markedly in their ability to generate APC in response to elevated thrombin levels is intriguing and may have important therapeutic implications. The results suggest that depending on individual defects in the protein C pathway, some patients may have impaired protein C activation and might require APC therapy, whereas others may benefit from administration of inactive protein C. The theoretical advantage of using APC rather than its inactive precursor protein C as replacement therapy is that APC is already in an active form and will be effective even in individuals in which conversion of protein C to APC is impaired due to decreased levels of TM and EPCR on the vascular endothelium. The theoretical advantage of using protein C is that it is converted to APC on site and on demand at sites of increased thrombin generation, and APC generation ceases once thrombin generation is controlled by endogenous anticoagulant mechanisms. The clinical efficacy of APC in severe sepsis has been demonstrated in a randomized, placebo-controlled phase 3 clinical trial. In contrast, the efficacy of protein C in such patients is unknown. Recently, a phase 2 study was conducted to assess the dosing regimen of protein C concentrate in 40 children with severe meningococcal sepsis. The children were randomized to receive either placebo or protein C concentrate (200 IU/kg, 400 IU/kg, or 600 IU/kg) for a maximum of 7 days. Sustained increases in plasma APC were observed in patients who received the highest dose. A larger trial in children, currently under way, will be needed to determine whether protein C infusion demonstrates a survival benefit.

The second question that we addressed in this study is whether markers of endogenous APC generation correlate with 28-day all-cause mortality. We identified no statistically significant difference between survivors and nonsurvivors in terms of baseline protein C levels (Figure 2; Table 3). This finding is consistent with a study of 70 patients with severe sepsis by Yan et al, which reported that there was no statistically significant difference

Table 4. Baseline levels of sEPCR and sTM in patients with severe sepsis and in healthy volunteers

<table>
<thead>
<tr>
<th>Variable</th>
<th>Survivors (n = 32)</th>
<th>Nonsurvivors (n = 18)</th>
<th>Healthy volunteers (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Min, max</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>sEPCR (ng/mL)</td>
<td>328.8 (95.1)</td>
<td>168.7, 509.5</td>
<td>290.2 (100.8)</td>
</tr>
<tr>
<td>sTM (ng/mL)</td>
<td>122.1 (425.9)</td>
<td>2.8, 1601.7</td>
<td>26.0 (72.9)</td>
</tr>
</tbody>
</table>

Data are summarized using means, standard deviations (SD), minimums (min), and maximums (max).
between survivors and nonsurvivors with respect to baseline protein C levels (P = .19). The authors did observe a statistically significant difference between survivors and nonsurvivors in terms of protein C levels measured at 44 hours (P = .04). We also observed no statistically significant differences between survivors and nonsurvivors in baseline APACHE II and F1 + 2 (Figure 2; Table 3). In contrast, baseline APC levels in survivors were statistically significantly higher than in nonsurvivors (P = .024) (Figure 2; Table 3). In addition, baseline F1 + 2/ APC ratios in survivors were statistically significantly lower than in nonsurvivors (P = .047) (Figure 2; Table 3). These results suggest that baseline APC levels and/or the F1 + 2/2–to-APC ratios may have prognostic value in predicting clinical outcome, although larger studies are needed to confirm or refute this observation. Consistent with this observation, de Kleijn et al40 reported that in children with severe meningococcal sepsis, the baseline thrombin-antithrombin (TAT)–to-APC ratios in survivors was statistically significantly lower than in nonsurvivors (P = .02).

Severe sepsis is an extremely heterogeneous condition, and the factors involved in down-regulation of the endothelium-based protein C activation complex in a subpopulation of patients remain to be identified. Our preliminary results suggesting that baseline APC levels and/or the F1 + 2/2–to-APC ratios may have prognostic value in predicting clinical outcome will be validated in future logistic regression analyses with a larger sample size to determine if the APC levels and/or the F1 + 2/2–to-APC ratios are dependent on events and exposures in the ICU such as the site and type of infection, the use of mechanical ventilation, and whether the patients had malignancies. Preliminary analyses of these 32 patients indicate that there is no significant correlation between age and plasma APC levels (r = 0.10, P = .59) and F1 + 2/2–to-APC ratios (r = 0.035, P = .85). We did observe suggestive trends that lung infection might contribute to high F1 + 2/2–to-APC ratios. We found that the lung was the primary site of infection in 6 of the 7 nonsurviving patients with the highest F1 + 2–to-APC ratios (ie, 86%). The overall incidence of lung infection in survivors and nonsurvivors was 43% and 72%, respectively.

In summary, future studies with a larger sample size may establish if baseline APC levels or baseline F1 + 2–to-APC ratios have prognostic value in predicting clinical outcome. If so, they may provide a quick and cost-effective means of identifying those patients who will benefit most from recombinant APC infusion. This is theoretically feasible since the APC assay that we have developed permits, for the first time, both rapid and accurate measurements of plasma APC levels.

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References


