Activated protein C for the treatment of severe sepsis

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Abstract

In 2001, the PROWESS (Recombinant Human Activated Protein C Worldwide Evaluation in Severe Sepsis) trial demonstrated a 6.1% absolute decrease in mortality in patients with severe sepsis. Recombinant human activated protein C was subsequently licensed for use by both the US Food and Drug Administration and the European Medicines Evaluation Agency. There has been some controversy over aspects of the original study protocol, and subsequent trials have raised concerns about both the efficacy and the side effect profile of recombinant human activated protein C. Significant doubt remains as to the role of recombinant human activated protein C in the management of severe sepsis, and this review aims to summarize the evidence both for and against its use.

Keywords: Drotrecogin alfa (activated), recombinant activated protein C, review, sepsis, sepsis treatment, severe sepsis, xigris


Introduction

Severe sepsis is an increasingly common condition that is associated with high levels of morbidity and mortality that place a large burden upon healthcare resources throughout the world. In the USA, severe sepsis accounts for approximately 215,000 deaths annually, equal to the number of deaths from myocardial infarction, with estimated annual costs of $16.7 billion [1]. The incidence of sepsis has increased over the past two decades [2] and, with an ageing population, is likely to continue to rise. Despite many advances in critical care over the years, the level of mortality due to sepsis remains high, and although there has been a modest reduction in the mortality rate, the actual number of deaths due to severe sepsis continues to rise [2]. Furthermore, for those who do survive, quality of life is frequently significantly diminished [3].

In 2001, the PROWESS trial of recombinant human activated protein C (APC) (drotrecogin alfa activated, hereafter referred to as drotrecogin alfa) for severe sepsis reported a 6.1% absolute reduction in mortality and a 19.4% relative reduction in the risk of mortality at 28 days [4]. Since the publication of the results of this trial, there has been, and continues to be, much debate over the efficacy and safety of this expensive therapy.

The Pathophysiology of Sepsis and the Role of APC

Sepsis is the systemic response by a host organism to invasion by a pathogenic microorganism, resulting in activation of inflammatory and coagulation pathways and inhibition of fibrinolysis. The dysregulation of these pathophysiological mechanisms significantly contributes to the organ dysfunction observed in severe sepsis.

The initial response by the host to invasion by a pathogenic microorganism is initiated by toll-like receptors, which are pattern recognition receptors that recognize specific cell wall molecules within bacterial, viral and fungal cell walls [5]. It is in response to recognition of the microbial pathogen by the toll-like receptors that the complex host immune response is triggered. Initially, proinflammatory mediators such as interleukin-1, interleukin-6, tumour necrosis factor alpha, nitric oxide, prostaglandins and other acute-phase proteins are released. Among other activities, these mediators induce leukocytes, upregulate tissue factor, and activate the coagulation pathways. Concurrently, there is depletion of endogenous anticoagulants such as antithrombin, heparin and APC, as well as inhibition of normal fibrinolysis. The activation of the coagulation cascade, together with depletion of endogenous anticoagulants and impaired fibrinolysis, results
in the generation of thrombi within the microcirculation, which can lead to tissue hypoxia and organ dysfunction.

Following the initial marked inflammatory response to sepsis, there is a sustained anti-inflammatory or immunosuppressive status. A major inflammatory control mechanism during this phase is apoptosis of immune effector cells such as B-lymphocytes, T-lymphocytes, and dendritic cells. There are two main mechanisms by which apoptosis impairs the immune response. The first mechanism leads to a profound decrease in B-cell and T-cell numbers, resulting in impairment of the adaptive immune response. This also impairs the innate immune response because of important cross-talk that occurs between the innate and adaptive immune systems [6].

The second mechanism by which apoptosis results in immune dysfunction is induction of immune unresponsiveness, or anergy, in the surviving immune cells. Clinical studies of patients with sepsis demonstrate that the degree of apoptosis of circulating lymphocytes correlates with the severity of sepsis and predicts fatal outcome in septic shock, suggesting the importance of apoptosis as a biomarker [7–9].

Given the extent of the problem that sepsis poses, it is of little surprise that much time and many resources have been invested in searching for treatments for this devastating condition. Given that the initial trigger appears to be overstimulation of the inflammatory cascade and resulting coagulation dysfunction, many agents targeting specific mediators of the inflammatory and coagulation cascades have been investigated. Some agents studied include corticosteroids, tumour necrosis factor antagonists, interleukin-1 antagonists, anti-endotoxin antibodies, and antithrombin. Unfortunately, none has led to improvement in survival rates, and, indeed, in some situations, they may even cause harm [10].

**APC**

APC is an endogenous protein that acts as an important modulator of both the inflammatory and coagulatory responses associated with severe sepsis. APC has anticoagulant, anti-inflammatory, pro-fibrinolytic and antiapoptotic effects. Several studies have shown that low levels of circulating protein C are associated with increased morbidity and mortality [11–13]. In addition to baseline levels of protein C, early changes in protein C levels are also predictive of outcome in patients with severe sepsis [14]. Protein C is activated on the endothelial surface by the thrombin–thrombomodulin complex to yield APC. APC inactivates factor Va and factor VIIIa, thereby blocking the amplification of the coagulation system; this process is accelerated by the cofactor protein S. APC has anti-inflammatory effects, by inhibiting the formation of tumour necrosis factor, interleukin-6, and interleukin-8, as well as by inhibiting neutrophil chemotaxis. APC promotes fibrinolysis by binding and inhibiting plasminogen activator inhibitor-1, a potent antifibrinolytic factor, and hence acts indirectly as a profibrinolytic agent. Finally, APC has also been shown to possess antiapoptotic properties, and this mechanism has been shown to be neuroprotective in stroke models [15,16]. These properties differ from those of tissue factor pathway inhibitor and antithrombin, two other anticoagulants, both of which failed to reduce mortality in large trials in severe sepsis [17,18].

In 2001, the results of the PROWESS trial were published. It was a randomized, double-blind, placebo-controlled, multicentre trial involving 164 centres in 11 countries. It enrolled 1690 patients with severe sepsis of less than 24 h duration to receive either drotrecogin alfa or placebo. There was a large number of exclusion criteria, mainly aimed at reducing the risk of excessive bleeding.

There was good adherence to the study protocol, and the patients were heterogeneous. Enrolment was stopped early at the second interim analysis, because the difference in mortality rates between the two groups exceeded the a priori guideline for stopping the trial. Drotrecogin alfa was associated with a 19.4% reduction in the relative risk of death and an absolute reduction in the risk of death of 6.1% (p 0.005). The incidence of serious bleeding showed a trend towards being higher in the drotrecogin alfa group (3.5% vs. 2.0%; p 0.06) [4].

Given the high purchase cost of the drug and the potentially large eligible patient group, the healthcare resource implications were huge, and triggered subgroup analyses of the PROWESS dataset, with a view to targeting those who may most benefit from drotrecogin alfa. Notwithstanding the inherent difficulties of post hoc subgroup analysis, it was not shown that the groups with the most severe disease, according to either APACHE II score (Table 1) or number of organ failures (Table 2), received the largest benefit from treatment with drotrecogin alfa [19].

In November 2001, the US Food and Drug Administration (FDA) approved the use of drotrecogin alfa for those with an APACHE II score ≥25. In 2002, the European Medicines Evaluation Agency (EMEA) approved its use for those with multiple organ failure. The FDA mandated that studies be undertaken to further evaluate the efficacy and safety of drotrecogin alfa in certain patient groups, including those with severe sepsis and a low risk of death, and paediatric populations. The FDA also mandated a study to investigate medium-term survival of those enrolled in the PROWESS trial.

The Administration of Drotrecogin Alfa (activated) in Early Stage Severe Sepsis (ADDRESS) Study [20] attempted
TABLE 1. Mortality outcomes in subgroups according to APACHE II score and treatment effect in PROWESS

<table>
<thead>
<tr>
<th>Population</th>
<th>Activated protein C</th>
<th>Placebo</th>
<th>Absolute risk reduction</th>
<th>Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APACHE II (quartiles)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st (3–19)</td>
<td>33/218 (15.1)</td>
<td>26/215 (12.1)</td>
<td>–3.0</td>
<td>1.25 (0.97–2.02)</td>
</tr>
<tr>
<td>2nd (20–24)</td>
<td>49/218 (22.5)</td>
<td>57/222 (25.7)</td>
<td>3.2</td>
<td>0.88 (0.63–1.22)</td>
</tr>
<tr>
<td>3rd (25–29)</td>
<td>48/204 (23.5)</td>
<td>58/162 (35.8)</td>
<td>12.3</td>
<td>0.66 (0.48–0.91)</td>
</tr>
<tr>
<td>4th (30–53)</td>
<td>80/210 (38.1)</td>
<td>118/241 (49.0)</td>
<td>10.9</td>
<td>0.78 (0.63–0.96)</td>
</tr>
<tr>
<td>APACHE II (higher vs. lower)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st/2nd quartiles</td>
<td>82/436 (18.8)</td>
<td>83/437 (19.0)</td>
<td>0.2</td>
<td>0.99 (0.75–1.30)</td>
</tr>
<tr>
<td>3rd/4th quartiles</td>
<td>128/414 (30.9)</td>
<td>176/403 (43.7)</td>
<td>12.8</td>
<td>0.71 (0.59–0.85)</td>
</tr>
</tbody>
</table>

TABLE 2. Subgroups according to number of organ failures and response to treatment in PROWESS

<table>
<thead>
<tr>
<th>Number of organ dysfunctions</th>
<th>Death in group, n (%)</th>
<th>Drotrecogin alfa</th>
<th>Placebo</th>
<th>Absolute risk reduction</th>
<th>Relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42/216 (19.4)</td>
<td>43/203 (21.2)</td>
<td>1.8</td>
<td>0.92 (0.63–1.34)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>56/270 (20.7)</td>
<td>71/274 (25.9)</td>
<td>5.3</td>
<td>0.80 (0.59–1.08)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>56/214 (26.2)</td>
<td>75/217 (34.6)</td>
<td>8.4</td>
<td>0.76 (0.57–1.02)</td>
<td></td>
</tr>
<tr>
<td>≥4</td>
<td>56/150 (37.3)</td>
<td>70/146 (47.9)</td>
<td>10.6</td>
<td>0.78 (0.60–1.02)</td>
<td></td>
</tr>
<tr>
<td>Single vs. multiple organ dysfunction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>42/216 (19.4)</td>
<td>43/203 (21.2)</td>
<td>1.8</td>
<td>0.92 (0.63–1.34)</td>
<td></td>
</tr>
<tr>
<td>≥2</td>
<td>168/634 (26.5)</td>
<td>216/637 (33.9)</td>
<td>7.4</td>
<td>0.78 (0.66–0.93)</td>
<td></td>
</tr>
</tbody>
</table>

Controversies in the Use of APC

Since the original publication of the PROWESS trial, there has been, and continues to be, much debate about the use of drotrecogin alfa. There remains some unease in some quarters of the clinical and scientific communities about its use, particularly with regard to the original study protocol and the efficacy and safety profile of drotrecogin alfa [23–25].

Debate has centred on protocol changes during the original PROWESS study. In the early stages of the trial, an external evaluation committee advised that a large number of patients who were recruited had a high risk of death from causes other than sepsis. The protocol was amended after 720 patients had been recruited prior to the first interim analysis to exclude such subjects, resulting in patients with less severe comorbidity and more acute infectious disease. In addition, at approximately the same time, a new master cell bank was introduced, from which the drotrecogin alfa was manufactured. Both Eli Lilly and the FDA performed extensive tests on both batches of drotrecogin alfa, and no significant differences were found. Following these changes, the reduction in mortality associated with treatment with drotrecogin alfa was greater than it had been up to that point (Table 3).

The apparent improvement in efficacy could be due to a number of potential factors, including different patient populations, undetectable changes in the drug, chance, or a...
combination of these three factors. The FDA thoroughly investigated this in their review, and concluded that "The p value for the interaction between the trial results pre- and post-amendment is 0.08. While this p value suggests that the impact of changes in the trial should be looked into, it is also not consistent with chance variation. Analyses of various factors that might have led to outcome differences between the trial halves did not support concerns that any of the factors likely accounted for these differences. We have therefore concluded that the differences most likely did arise by chance" [19].

The use of the APACHE II scoring system in the trial has also been questioned [10]. The APACHE II is a severity of disease scoring system that combines physiological data with information on age and comorbidity as well as diagnosis [26]. It was designed and validated to predict mortality based on data from the first 24 h of intensive-care unit care, and is not seen as having appropriate accuracy to allow individual prognostication or indeed to guide therapy for individual patients. In addition, as an APACHE II score also takes into account other risk factors for death, such as age and comorbidity, the effect of using an APACHE II score to determine treatment would be to introduce bias against the elderly and chronically infirm. Despite the limitations of using APACHE II scores to decide upon individual treatment, an APACHE II score was shown to be the best discriminator of death in the PROWESS trial (Table 1), and was recommended by the FDA for the identification of high-risk patients with severe sepsis.

As shown above, the efficacy demonstrated by drotrecogin alfa in the PROWESS trial has not been reproduced in subsequent randomized controlled trials in paediatric populations or in those with sepsis and a low risk of death [20,21]. A post hoc subgroup analysis of the small (321) number of patients included in the ADDRESS study with an APACHE II score ≥25 failed to show benefit of drotrecogin alfa. The mortality rate was higher, albeit not statistically significantly, among the group receiving drotrecogin alfa than in the placebo group (29.5% vs. 24.7%). Again, this was a post hoc subgroup analysis, with all of the inherent limitations associated with this. It should be noted that, when compared with those of the PROWESS study, these subjects had a lower mean APACHE II score, and their mortality rate was significantly lower than the 43.7% observed in the placebo group of the PROWESS study. This may simply reflect the fact that, as intended by the design of the study, less severely ill patients were enrolled in the ADDRESS study, which also demonstrates some of the inherent limitations of using APACHE II scores to predict outcome in individual patients.

Concerns regarding the increased incidence of serious bleeding events also persist. A large, multicentre, multinational, non-randomized, open-label trial of drotrecogin alfa involving 2378 patients (the ENHANCE trial), which had inclusion and exclusion criteria similar to those of PROWESS, was able to demonstrate 28-day mortality similar to that achieved in PROWESS, but also had a higher incidence of serious bleeding events (6.5% vs. 3.5%). The authors speculate that there may have been a higher background bleeding rate in the subjects of this study. They have based this speculation on the increased incidence of post-infusion bleeding (3.2% vs. 1.2%) "presumably when the drotrecogin alfa infusion effect would be gone", coupled with the higher number of surgical patients and the higher haematological and hepatic SOFA scores in these subjects. This same study also showed that patients with severe sepsis treated with drotrecogin alfa within the first 24 h of their organ dysfunction exhibited lower mortality, regardless of disease severity, and the authors suggested that time to treatment could be assessed in future trials [27].

**To Which Patients should Drotrecogin Alfa be Targeted?**

It can be said with relative certainty that the following groups should not receive drotrecogin alfa:

1. Children
2. Patients with severe sepsis and a low risk of death
3. Patients with severe sepsis and single-organ failure
4. Patients with exclusion criteria matching those of the original PROWESS study, including those who underwent major surgery within the preceding 12 h.

Given the uncertainty of the benefit of this expensive therapy, coupled with the increased risk of serious bleeding events, the decision concerning who should receive treatment remains difficult. Currently, there is no consensus on this matter within the critical-care community. Both the FDA and the EMEA recommend that drotrecogin alfa be used only in patients at high risk of death due to sepsis. The FDA specified an APACHE II score of ≥25 and the EMEA specified acute multi-organ failure, when therapy can be started within 24 h of the onset of organ

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**TABLE 3. Changes in mortality rates before and after the protocol amendment**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number</th>
<th>Placebo group</th>
<th>Treatment group</th>
<th>Relative risk of death</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before amendment</td>
<td>720</td>
<td>30</td>
<td>28</td>
<td>0.94</td>
<td>0.57</td>
</tr>
<tr>
<td>After amendment</td>
<td>970</td>
<td>31</td>
<td>22</td>
<td>0.71</td>
<td>0.001</td>
</tr>
</tbody>
</table>
failure. Others have sensibly suggested that the decision should be made by experienced clinicians, taking into consideration all the clinical data, to assess the chances of improved survival vs. the risk of serious bleeding [28,29]. Although this is a sensible suggestion, we do know that the ability of critical-care doctors to predict the risk of death is limited [30]. A recently published review by the Cochrane Collaboration concluded that "there was no evidence suggesting that drotrecogin alfa should be used for treating patients with severe sepsis or septic shock. Additionally, drotrecogin alfa seems to be associated with a higher risk of bleeding. Unless additional RCT’s provide evidence of a treatment effect, policy-makers, clinicians and academics should not promote the use of drotrecogin alfa” [31].

It is of interest for the future that research looking at chemically altering the APC molecule to alter its antiocoagulant effects, while maintaining its anti-inflammatory effects, has recently been instigated, but this is still at a very early stage of development [32].

Conclusions

Drotrecogin alfa remains the only drug that has been proven in a well-conducted randomized controlled trial to decrease mortality in patients with severe sepsis. Despite this, further studies have raised concerns regarding both the efficacy and the side effect profile of this agent, such that significant doubt exists as to its role in the management of severe sepsis. A study of plasma APC levels, drotrecogin alfa dose and outcome is underway. In addition, a further randomized trial is currently underway that is attempting to demonstrate whether patients can be prospectively identified and benefit from treatment with drotrecogin alfa. Following these trials, we will hopefully have clearer evidence of its usefulness in this devastating condition. Until then, clinicians should use this drug only after careful consideration of the risks and benefits.

Transparency Declaration

G. Houston declares no competing interests. B. H. Cuthbertson has received consulting fees, grant support and lecture fees from Eli Lilly and Co.

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