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Drotrecogin Alfa (Activated)

Treatment of Septic Shock

LY-203638
rhAPC
Xigris™
Zovant™ (former brand name)

Recombinant human activated protein C

EN: 275685

Introduction

Sepsis is a syndrome characterized by an extreme systemic response to infection which can quickly lead to multiple organ failure and death. Sepsis is the cause of death in more than 1400 people worldwide every day and each year over 700,000 new cases are diagnosed (1). Of those individuals suffering from sepsis, 30% die within the first months while up to 50% die within 6 months (2, 3). In general, sepsis develops from pneumonia. trauma, surgery, burns, cancer or AIDS-related infection. Critically ill hospitalized patients are often victims although it can affect anyone. The term sepsis encompasses a range of conditions that are massive immune responses to infection or trauma. More specifically, the immune response includes systemic inflammation and abnormal clotting and bleeding that leads to organ failure and death (4).

Sepsis develops in individuals with evidence of infection and the most frequent sites of infection in these patients are the lungs, abdomen, urinary tract and pelvis (5). The diagnosis of sepsis is difficult. The accepted diagnostic criteria begins with evidence of infection in addition to presentation of any two of the following: a heart rate faster than 90 beats/min, increased respiratory effort, high or low white blood cell count and/or high or low body temperature (5). In the presence of infection, sepsis symptoms present as reduced mental alertness, confusion, shaking, chills, fever, nausea, vomiting and diarrhea. The outcome of sepsis varies. Some patients rapidly develop septic shock where the cardiovascular system begins to fail while others experience differential organ dysfunction or they begin to recover (6).

The actual mechanism of sepsis development remains unclear and there are currently no treatments available specifically for the treatment of sepsis. Although inflammation is a critical factor, antiinflammatory agents (*e.g.*, ibuprofen and antitumor necrosis factor [TNF]) fail to prevent death in patients suffering from sepsis. Evidence suggests that coagulation and fibrinolysis may be key elements in sepsis development (4). Because the cause of sepsis continues to elude investigations, treatment only involves managing the underlying infection and treatment to support organ dysfunction.

However, recent evidence suggests that activated protein C, a component of the body's own anticoagulant system, may play an important role in the inflammation and clotting observed in sepsis. Activated protein C is a potent antithrombotic serine protease with antiinflammatory properties. It inhibits activated factors V and VII, thus reducing thrombin formation and stimulates fibrinolysis via suppression of plasminogen-activator inhibitor type 1 levels. Moreover, it has been demonstrated that the majority of patients with sepsis have reduced levels of activated protein C and studies have shown that administration of activated protein C to baboons with Gram-negative sepsis enhanced survival (7-9).

Researchers at Eli Lilly have developed a recombinant version of endogenous activated protein C, drotrecogin alfa (activated) (rhAPC; Xigris™, formerly Zovant™), that was chosen for further development for the treatment of sepsis.

Pharmacological Actions

To avoid using human plasma as a source of activated protein C, several articles and patents have been published presenting various procedures to produce high levels of functional recombinant human activated protein C (rhAPC) in various cell lines (10-14).

In an effort to define the mechanism of proinflammatory regulation of rhAPC, a study incorporating transcript profiling, flow cytometry and Western blotting of human umbilical vein endothelial cells (HUVEC) treated with rhAPC revealed that the agent dose-dependently

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inhibited the expression of the NFκB pathway and down-regulated expression of ICAM-1, VCAM-1, E-selectin and fractalkine (15). Another *in vitro* study further examining the mechanism of action of rhAPC demonstrated that incubation with the agent (10 nM) resulted in complete inactivation membrane-bound human factor VII after 4 h of incubation and complete inactivation of human plasma factor V after only 5 min of incubation. When rhAPC was combined with human plasma protein S (100 nM), inactivation of human recombinant factor VII and plasma factor Va was enhanced by 6.4- and 2-fold, respectively; rhAPC-induced inactivation of membrane-bound factor V was similar in the presence and absence of protein S. Results show that factors VII, V and Va are important physiological substrates for rhAPC-induced inaction (16).

The anticoagulant properties of rhAPC were elucidated in an *in vitro* study using human plasma. The agent (0.2 μg/ml) was found to prolong the activated partial thromboplastin time (APTT) assay by 2 times; rhAPC only had slight effects or no effects on recalcification and prothrombin time assays. rhAPC at concentrations of 0.3, 0.4, 0.5, 0.6 and 1 μg/ml was also found to double APTT assays using blood/plasma from monkey, dog, cat, rabbit and rat, respectively; the action of the recombinant human form of the agent was comparable to that of plasma-derived APC (17).

Studies in a rat model of carotid artery thrombosis revealed that rhAPC (2.6 and 5.2 mg/kg i.v.) induced significant antithrombotic effects and prolonged APTT time; the agent had no effect on factor Vc or factor VIIIc. The half-life of rhAPC in rats was about 20 min (17).

Although the efficacy of rhAPC against sepsis is thought to be due to both anticoagulant and antiinflammatory actions, a study using rat models of acute inflammation (*i.e.*, the dermal reversed passive Arthus model and the intestinal ischemia/reperfusion model) did not demonstrate any antiinflammatory effects of the agent. Neither dermal nor lung neutrophil recruitment could be attenuated with rhAPC treatment (18).

The preclinical efficacy of rhAPC (1 and 2 mg/kg/h i.v. infusion over 2 h) was further demonstrated in a canine model of coronary artery thrombosis. The left circumflex coronary artery of animals was stimulated with 100 μA of direct current for 60 s to cause endothelial cell injury and subsequent thrombosis formation and complete occlusion. The agent was administered 15 min before electrical stimulation and anticoagulant and antithrombotic effects were assessed in *ex vivo* assays. rhAPC at doses of 1 and 2 mg/kg/kg significantly increased APTT 2- and 3.7-fold, respectively, over the 2-h infusion time; no effect on thrombin time was noted. In addition, significant prolongations to occlusion of 186 ± 21 and 190 ± 22 min were observed with the respective doses as compared to controls (86 ± 12 min) and in animals treated with the 0.5 mg/kg/h dose (93 ± 17 min). Vessel patency was improved in 3 of 6 vessels and 3 of 5 vessels from groups treated with 1 and 2 mg/kg/h rhAPC, respectively, as compared to controls (0/5 vessels) and the 0.5 mg/kg/h group (0/6 vessels). Template bleeding times were significantly increased only in groups treated with 1 mg/kg/h with peaks observed 60 min into the infusion. No alterations in platelet aggregation were detected in any of the treatment groups (19).

Results from a study using a nonthrombotic canine model of myocardial reperfusion injury indicated that rhAPC had no cardioprotective activity when administered at an infusion rate comparable to that which inhibits coagulation in dogs (0.3, 1 and 3 μg/ml). Treatment did result in inhibition of thrombin generation although no antiinflammatory effects were observed (20).

In vivo experiments conducted in rhesus monkeys with preformed jugular venous thrombi showed that rhAPC (60 μg/kg bolus followed by 15 μg/kg/h for 2 h) prevented [^{125}I]-fibrinogen accretion. Accretion of [^{125}I]-fibrin to jugular venous clots was significantly less in rhAPC-treated animals as compared to untreated controls (4.7 ± 6.2 *vs.* 55.4 ± 33.4 mg/thrombus). No significant changes in bleeding were observed with treatment. No marked changes in APTT time and no changes in prothrombin time, factor V or factor VII levels were observed. Similar results were obtained in dogs with preformed jugular venous thrombi treated with purified plasma-derived APC (21).

An optimized immunocapture-amidolytic assay has been described for the quantification of rhAPC. This optimized in vitro assay increased the V_{max} for the agent more than 10-fold. Sensitivity was 2.5 ng/ml. The assay may be used for determining human plasma concentrations of the agent (22).

Clinical Studies

The efficacy of rhAPC (12 or 18 [low-dose] or 24 or 30 [high-dose] μg/kg/h for 48 or 96 h) was demonstrated in a prospective, randomized double-blind, placebo-controlled phase II trial of 131 patients with severe sepsis. Upon entry, all patients displayed D-dimer levels above normal, indicating the presence of microvascular coagulopathy, and 92% were protein C deficient. High-dose rhAPC significantly and dose-dependently decreased D-dimer. In addition, a trend toward improvement in Systemic Inflammatory Response Syndrome (SIRS)-, ICU- and hospital-free days was observed in the high-dose group as compared to placebo. The 28-day mortality was decreased to 21% in the high-dose groups as compared to 35 and 34% in the low-dose group and placebo, respectively. There was a 40% relative risk reduction seen in the high-dose group although this was not statistically different from placebo (23).

Another multicenter phase II trial conducted in patients with severe sepsis obtained similar results and was the basis for phase III trials. Trends toward improvements in SIRS-, shock-, ventilator-, ICU- and hospital-free days and decreases in IL-6 levels were observed with rhAPC treatment (24).

Results from a prospective, multicenter, randomized, double-blind, placebo-controlled trial involving 1690

Box 1: Efficacy and safety of drotrecogin alfa in patients with severe sepsis (26) [Prous Science CSline database].

Design	Randomized, double-blind, placebo-controlled, multicenter clinical study
Population	Patients with severe sepsis (n = 1690)
Treatments	Drotrecogin, 24 μg/kg/h i.v. infusion over 96 h (n = 850) Placebo (n = 840)
Adverse events	D: 106/850 (12.5%) [serious bleeding event 30/850 (3.5%), thrombotic events 17/850 (2.0%)] P: 102/840 (12.1%) [thrombotic events 25/840 (3.0%), serious bleeding event 17/840 (2.0%)]
Results	Death rate @ 28 d: P (259/840 [30.8%]) > D (210/850 [24.7%]) [$p = 0.005$] Plasma D-dimer levels (mg/ml), change @ 7 d: D (–0.5) > P (0.25) [$p = 0.014$]
Conclusions	Activated drotrecogin alfa reduced mortality in patients with severe sepsis, although it appeared to increase the bleeding risk

patients with severe sepsis presenting systemic inflammation (3-4 criteria for SIRS) and organ failure due to acute infection, showed the efficacy of rhAPC (24 μg/kg/h continuous infusion for 96 h). Twenty-eight days after the start of treatment, significantly fewer rhAPC-treated patients died as compared to placebo (30.8 *vs.* 24.8%). Moreover, rhAPC treatment was associated with a decrease in the relative risk of death of 19.4% and a significant absolute reduction in the risk of death of 6.1%. rhAPC-treated patients also displayed significantly lower D-dimer levels on days 1-7 postinfusion as compared to placebo. In addition, significantly greater decreases in IL-6 levels were seen on days 1, 4, 5, 6 and 7 as compared to placebo. Both treatment and placebo groups had a similar percentage of patients experiencing at least 1 serious adverse event (12.5 and 12.1%, respectively) and the incidence rates of thrombotic events and new infection were similar in both groups. However, a higher incidence of serious bleeding was noted in the rhAPC-treated group as compared to placebo (3.5 *vs.* 2%; $p = 0.06$). Serious bleeding was only observed during the infusion period, after which the incidence was similar for both treated and placebo groups. No neutralizing antibodies against rhAPC were detected in any of the patients (25, 26) (Box 1).

Mortality data from the PROWESS study involving 1690 patients with severe sepsis who were treated with rhAPC at a dose of 24 μg/kg/h for 96 h or placebo, has recently been reported. In the intent-to-treat population, a significant reduction in 28-day all-cause mortality was observed in the rhAPC patients, with a relative risk reduction of 19.4%. Moreover, a survival benefit was seen in a variety of patient subpopulations defined by various demographic and historical characteristics. A highly significant treatment benefit was seen in patients without a history of myocardial infarction (relative risk reduction of 27%). A consistent treatment benefit was also seen in subgroups of patients defined by baseline protein C activity, antithrombin activity and IL-6 levels. Significant reductions in mortality were also seen regardless of age, although patients younger than 65 years of age or 75 years of age and older appeared to derive more benefit from rhAPC, with relative risk reductions of 26% and 32%, respectively, *versus* 19% for those 65 years of age and older. Further analysis of data from this trial demonstrated that patients treated with rhAPC had more rapid resolution of coagulopathy and inflammation compared to those given placebo. Finally, an analysis of the costs associated with rhAPC treatment in these patients indicated that the survival benefit is not associated with increased hospital costs or resource use over the first 28 days (27-31).

rhAPC may also be effective against other disorders and a number of patents have been submitted in this regard. rhAPC has been proposed to be effective against heparin-induced thrombocytopenia; hemolytic syndrome; acquired hypercoagulable states or acquired protein C deficiency associated with purpura fulmans, bone marrow and other transplantations, severe burns, pregnancy, surgery, severe trauma, ARDS, sepsis and meningococcal sepsis; vascular occlusion and thromboembolic disorders including stroke, venous thrombosis, myocardial infarction, unstable angina, abrupt closure following angioplasty or stent replacement and thrombosis as a result of peripheral vascular surgery (32-36).

Eli Lilly has applied for regulatory approval of rhAPC as a treatment for sepsis in the U.S., the European Union and Australia, and the FDA has recently granted a priority review for the agent (37, 38).

Manufacturer

Eli Lilly and Company (US).

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